AstraZeneca

Clinical Pharmacology Study Protocol

Drug substance	AZD6140
Edition No.	Final Version 1.0
Study code	D5130C05265
Date	

A Randomized, Double-Blind, Two-Period Crossover Study to Assess Safety, Tolerability, and Pharmacokinetics Following Repeated Doses of AZD6140 (400 mg od) and Digoxin (0.25 mg od) in Healthy Male and Female Volunteers

The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

Amendment No.	Date of Amendment	Local Amendment No.	Date of local Amendment

Administrative change No.	Date of Administrative Change	Local Administrative change No.	Date of local Administrative Change

ASTRAZENECA EMERGENCY CONTACT PROCEDURE

In the case of a medical emergency you may contact the Clinical Study Team Leader. If the Clinical Study Team Leader is not available, contact the Clinical Study Team Physician or the Clinical Study Team Drug Safety Physician at the AstraZeneca Research and Development site shown below.

Role in the study	**Name**	**Address and Telephone number**
Clinical Study Team Leader		
Clinical Study Team Physician		
Clinical Study Team Drug Safety Physician		

For further clarifications regarding:

- Procedures in case of medical emergency see Section 8.2.
- Procedures in case of overdose see Section 8.3.

PROTOCOL SYNOPSIS

A Randomized, Double-Blind, Two-Period Crossover Study to Assess Safety, Tolerability, and Pharmacokinetics Following Repeated Doses of AZD6140 (400 mg od) and Digoxin (0.25 mg od) in Healthy Male and Female Volunteers

Investigator

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Study centre(s), type and number of subjects planned

Approximately 20 healthy male and female (non-child bearing potential) subjects between 18 and 65 years inclusive will be randomized at a single center to ensure that at least 12 subjects complete the study.

Study period	**Phase of development**
Estimated date of first subject enrolled	Clinical Pharmacology (I)
Estimated date of last subject completed	

Objectives

Primary Objective:

- To compare the steady state pharmacokinetics of digoxin when administered alone and in combination with AZD6140

Secondary Objectives:

- To compare the steady state pharmacokinetics of AZD6140 when administered alone and in combination with digoxin
- To assess the safety and tolerability following co-administration of AZD6140 and digoxin

Study design

This will be a randomized, double-blind, two-period crossover study. In one study period, each subject will receive AZD6140 400 mg once daily (od) in the morning for 16 days. A loading dose of digoxin 0.5 mg (0.25 mg bid) will be administered on Day 6, and digoxin 0.25 mg od will be co-administered with AZD6140 in the morning on Days 7-14. Digoxin will be administered open label. In the other study period, each subject will receive AZD6140 placebo od in the morning for 16 days. A loading dose of digoxin 0.5 mg (0.25 mg bid) will be administered on Day 6, and digoxin 0.25 mg od will be co-administered with AZD6140 placebo in the morning on Days 7-14. There will be a minimum 2-week washout period between treatments.

Investigational product, dosage and mode of administration

- AZD6140 200 mg immediate release tablets
- AZD6140 placebo to match 200 mg tablets
- Digoxin 0.25 mg tablets (to be supplied by the Principal Investigator) open-label

Duration of treatment

In each study period, AZD6140 400 mg or placebo will be administered once daily for 16 days. Digoxin 0.25 mg will be co-administered with AZD6140 or placebo for 9 days (twice daily on Day 6; once daily on Days 7-14). There will be a minimum 2-week washout period between study periods. The order in which the subjects receive the AZD6140 or placebo will be randomized.

Outcome variables

- **Pharmacokinetic**

  Primary pharmacokinetic assessments will be $C_{ss,max}$ and AUC_{τ} of AZD6140 and digoxin.

  Additional assessments include:

  - Digoxin: t_{max}, $C_{ss,av}$, $t_{½}$, CL/F, $C_{ss,min}$ and Ae_{24h} (total amount unchanged drug excreted in urine during last dosing interval)
  - AZD6140: t_{max}, $C_{ss,av}$, CL/F
  - AR-C124910XX: $C_{ss,max}$, t_{max}, $C_{ss,av}$, and AUC_{τ}

- **Safety**

  Adverse events, electrocardiograms [ECGs] (12-lead and telemetry), blood pressure, pulse, physical examination and laboratory assessments (chemistry, hematology, and urinalysis parameters)

- **Pharmacodynamic**

  Not applicable

- **Pharmacogenetics**

  Genetic analysis of the genes that are involved in the disposition of and response to AZD6140, such as the MDR-1 and CYP3A genes, will be performed for those subjects providing separate informed consent. The $P2Y_{12}$ receptor may also be examined.

  This genetic research component will provide data for possible retrospective analysis. Any result will not form part of the main study database nor the clinical study report. Ethical approval is required for this genetic research component, in addition to, and separate from, the approval of the main study protocol. Subject participation is voluntary and requires provision of an additional informed consent to participate in the main study.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study protocol.

Abbreviation or special term	Explanation
AE	Adverse event
Ae_{24h}	Cumulative amount of unchanged drug excreted into urine during the last dosing interval
ALT	Alanine aminotransferase
AMOS	Astra monitoring system
aPTT	Activated partial thromboplastin time
Assessment	An observation made on a variable involving a subjective judgement
AST	Aspartate aminotransferase
AUC	Area under plasma concentration-time curve from zero to infinity
$AUC_{(0-24)}$	Area under plasma concentration-time curve from zero to 24 hours
AUC_{τ}	Area under plasma concentration-time curve during a dosing interval
bid	Twice daily
BMI	Body mass index
C_{max}	Maximum plasma (peak) drug concentration after single dose administration
$C_{ss,av}$	Average drug concentration in plasma during a dosing interval at steady state on administering a fixed dose at equal dosing intervals
$C_{ss,max}$	Maximum (peak) steady state drug concentration in plasma during dosing interval
$C_{ss,min}$	Minimum (trough) steady state drug concentration in plasma during dosing interval
CL/F	Apparent oral clearance
CRF	Case report form
CV	Coefficient of variation
DCF	Data clarification form
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
g	Centrifugal force
GCP	Good Clinical Practice

Abbreviation or special term	Explanation
GGT	Gamma glutamyltransferase
GRand	Global randomization tool
HBsAg	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
λ_z	Smallest (slowest) disposition rate constant
Measurement	An observation made on a variable using a measurement device
MedDRA	Medical dictionary for regulatory activities
OAE	Other significant adverse event (See Appendix B)
od	Once daily
OTC	Over-the-counter
Outcome variable	A, usually derived, variable specifically defined to be used in the analysis of a study objective
Parameter	A quantity (usually unknown) that characterizes the distribution of a variable in a population of subjects
PD	Pharmacodynamic
Pgp	P-glycoprotein
PK	Pharmacokinetic
Principal investigator	A person responsible for the conduct of a clinical study at a study site. Every study center has a principal investigator.
PT	Prothrombin time
RBC	Red blood cell
SAE	Serious adverse event
SOC	System organ class
$t_{½}$	Half-life
t_{max}	Time to reach peak or maximum concentration or maximum response following drug administration
τ	Dosing interval
Variable	A characteristic or a property of a subject that may vary eg, from time to time or between subject
WBC	White blood cell

1. INTRODUCTION

1.1 Background

1.1.1 AZD6140

Adenosine diphosphate (ADP) is an important mediator of platelet activation and aggregation through its binding to at least two distinct subtypes of purinoceptor, designated $P2Y_1$ and $P2Y_{12}$, found on platelets. Two ADP receptor antagonists, clopidogrel and ticlopidine have shown clear benefits for the reduction of clinical thromboembolic events in patients with atherosclerosis due to their ability to block the $P2Y_{12}$-receptor. However, since this blockade is irreversible and usually incomplete, the search continues for agents, which can further improve the clinical outcomes of these patients through improved efficacy or safety.

AZD6140 is a potent, selective $P2Y_{12}$-receptor antagonist (antiplatelet agent) being developed to reduce thromboembolic events in patients with atherosclerosis. It is orally active and does not require metabolic activation. Unlike clopidogrel and ticlopidine, which incompletely block the $P2Y_{12}$-receptor response in humans, pre-clinical studies indicate that AZD6140 can produce long-lasting and complete inhibition of ADP-induced platelet aggregation *ex vivo* following oral dosing.

Study SC-532-5239 evaluated the safety and tolerability of multiple ascending doses of 50 mg to 600 mg/day AZD6140 in healthy male and female subjects. The safety of AZD6140 was also compared with clopidogrel. In addition, the effect of food on the pharmacokinetics and pharmacodynamics (inhibition of platelet aggregation) of AZD6140 was studied. Once and twice daily dosing regimens of AZD6140 with total daily doses ranging from 50 mg to 600 mg administered for 5 days at each dose level (a total duration of 15 or 20 days) were studied. The pharmacokinetics of AZD6140 following multiple oral dosing was approximately linear over 50 mg to 600 mg. Maximum plasma concentrations (C_{max}) were reached within 2 to 4 hours after dose intake, and the mean terminal half-life ($t_{½}$) of AZD6140 ranged from 6 to 13 hours. The metabolite area under the plasma concentration-time curve (AUC) and C_{max} were about 35% of the corresponding parameters for AZD6140 and were approximately linear over 50 mg to 600 mg dosing of AZD6140. An exploratory food effect determination resulted in <20% increase in AZD6140 C_{max} and <31% increase in AZD6140 AUC. Food did not have an effect on metabolite C_{max} and AUC. Although area under the curve (AUC) values were somewhat higher following administration of AZD6140 with food, there was no obvious effect of food on pharmacodynamic (PD) response. Greater than 80% inhibition of platelet aggregation was observed at all doses studied. In terms of inhibition of platelet aggregation, twice-daily doses were superior to the equivalent total daily dose given every 24 hours. All total daily doses of AZD6140 above 200 mg were superior to once-daily doses of 75 mg clopidogrel in terms of PD response.

No serious adverse events (SAE) were observed in the 5 trials completed to date, which exposed healthy subjects to daily doses from 0.1 mg to 600 mg for periods of up to 20 days. Approximately 120 healthy subjects were entered into these trials, and 89 healthy subjects received at least 1 dose of AZD6140. Petechiae, tachycardia, and postural hypotension were the most common adverse events reported in some of these trials. However, the causal

relationship of these adverse events to AZD6140 is uncertain. Four subjects in Study SC-532-5256 (single 200 mg dose study in healthy subjects) reported rashes that were considered by the investigator to have a reasonable possibility of a causal relationship to study treatment. Two of these subjects were withdrawn because of this adverse event.

In the multiple ascending dose study SC-532-5239, 1 subject had an increase in liver transaminases of more than 3 times the upper limit of normal after 4 daily doses of 300 mg AZD6140, and was discontinued from the study. The transaminases returned to normal over 24 days. Another subject had a milder increase in transaminase after 4 twice-daily doses of 200 mg that improved despite continuation and increase in dose of AZD6140. There were no other laboratory findings, and no ECG, Holter monitor or vital signs findings of concern during any of the studies.

1.1.2 Digoxin

Digoxin is a commonly prescribed cardiac glycoside. Its principle actions are an increase in the force of myocardial contraction and a reduction in the conductivity within the atrio-ventricular node. Digoxin is most useful in the treatment of supraventricular tachycardias (especially atrial fibrillation) and in the treatment of mild heart failure.

Digoxin has a narrow therapeutic range (plasma levels of 0.8-2.0 ng/mL) and toxicity correlates with the elevated plasma concentration of the drug and on the sensitivity of the conducting system and myocardium. In clinical practice, digoxin plasma concentrations greater than 2 ng/mL (8-24 hours post-dose) have been associated with toxicity, such as cardiac rhythm disturbances, nausea and vomiting. However, toxicity has also been observed in patients with plasma concentrations less than 2 ng/mL. Factors that increase the risk of toxicity include advanced age, hypokalemia, hypomagnesemia and renal failure.

Digoxin has plasma protein binding of 25%, is excreted via renal filtration (50-70% of the given dose), has an absolute bioavailability of 60-80%, and a terminal half-life ($t_{½}$) of 36-48 hours (1.5-2 days). Other important adverse reactions include loss of appetite, diarrhea, abdominal pain, headache, fatigue, drowsiness, visual disturbance and bradyarrhythmias. Toxicity is managed by discontinuing therapy and correcting any plasma electrolyte abnormalities. Digoxin-specific immune antibody fragments are available for reversal of life-threatening overdosage.

1.2 Rationale

Digoxin is a known P-glycoprotein (Pgp, MDR1) transporter substrate and increased digoxin levels have been observed with concomitant administration of Pgp substrates and/or inhibitors such as verapamil and atorvastatin. It is likely that digoxin will be co-administered with AZD6140 in the intended target population and in-vitro studies indicate that AZD6140 is a substrate for Pgp. Because digoxin is a drug with a narrow therapeutic range, the effect of potential pharmacodynamic and pharmacokinetic interactions of AZD6140 on digoxin must be explored.

2. STUDY OBJECTIVES

2.1 Primary objective

The primary objective of this study is to compare the steady state pharmacokinetics of digoxin when administered alone and in combination with AZD6140.

2.2 Secondary objective(s)

The secondary objectives of the study are:

- To compare the steady state pharmacokinetics of AZD6140 when administered alone and in combination with digoxin
- To assess the safety and tolerability following co-administration of AZD6140 and digoxin

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design

This is a randomized, double-blind, two-period crossover study designed to assess the safety, tolerability and pharmacokinetics following repeated doses of AZD6140 400 mg once daily and digoxin 0.25 mg once daily in healthy male and female volunteers. Approximately 20 healthy male and female (non-child bearing potential) subjects between 18 and 65 years inclusive will be randomized at a single center in order to obtain evaluable data from at least 12 subjects.

Screening evaluations including medical/surgical/medication history, physical examination, clinical laboratory evaluations including virology screen, drugs of abuse screen, 12-lead ECG, and vital sign measurements will be performed during the 21-day period prior to randomization (Day -21 to Day -1).

Upon randomization into the study, each subject will receive one of the following treatments per study period, the order as determined by the randomization schedule:

> **Treatment A:** AZD6140 400 mg once daily in the morning for 16 days. A loading dose of digoxin 0.5 mg (0.25 mg bid) will be administered on Day 6, and digoxin 0.25 mg once daily will be co-administered with AZD6140 in the morning on Days 7-14
>
> **Treatment B:** AZD6140 placebo once daily in the morning for 16 days. A loading dose of digoxin 0.5 mg (0.25 mg bid) will be administered on Day 6, and digoxin 0.25 mg once daily will be co-administered with AZD6140 placebo in the morning on Days 7-14

All doses of study medication will be administered orally. There will be a minimum 2-week washout period between study periods. A blood sample for genetic analysis will be obtained pre-dose on Day 1 of Study Period 1.

Blood samples for the determination of concentrations of AZD6140 and its metabolite, AR-C124910XX, will be taken on Days 1 (pre-dose), 4, 5, 13 and 14. Blood samples for the determination of digoxin concentrations will be taken on Days 1 (pre-dose), 12, 13 and 14. Urine will be collected for pharmacokinetic analysis of digoxin on Day 1 (pre-dose) and Day 14. Safety evaluations including adverse event queries, vital sign measurements, 12-lead ECGs and clinical laboratory assessments will be assessed frequently during the study periods and at follow-up.

A follow-up visit will occur 7-14 days after Study Period 2.

Figure 1 Study flow chart

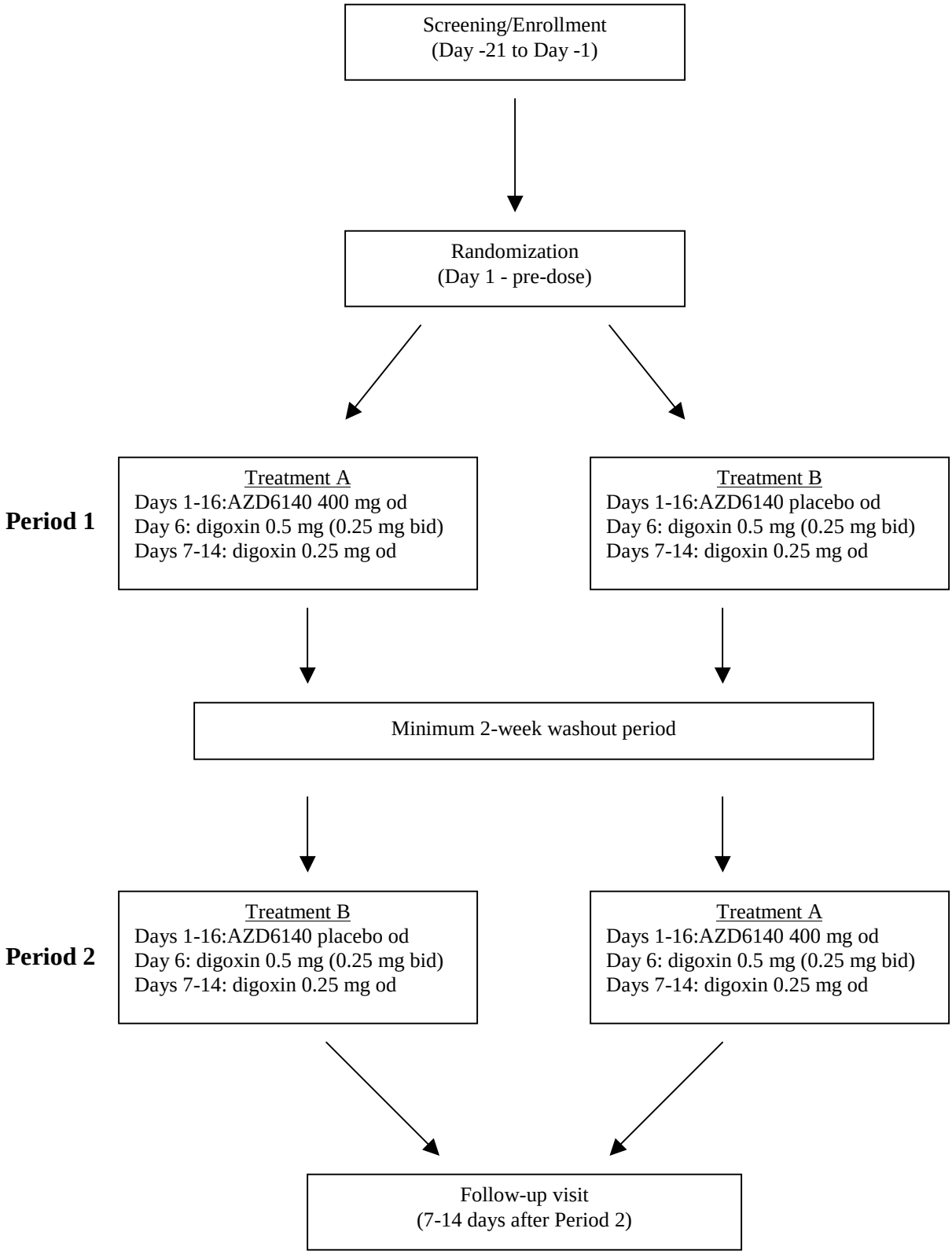


Table 1 Study plan

Study Day	Screening (Day -21 to –1)	Study Period 1 and 2																		Follow up[l]
		-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Informed consent	X																			
Demographics	X																			
Inclusion/exclusion criteria	X	X																		
Medical/surgical history	X	X																		
Medication history	X																			
Complete physical examination[a]	X																			X
Brief history and physical exam		X																	X	
12-lead ECG	X	X						X[k]	X[k]	X[k]	X[k]	X[k]	X[k]	X[k]	X[k]	X[k]	X[k]	X[k]	X	X
Vital signs[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Laboratory testing[c]	X	X		X[i]					X[i,j]								X			X
Stat digoxin levels[d]									X	X	X	X	X	X	X	X	X		X	
Pregnancy test[e]	X	X																		X
Alcohol/drugs of abuse screen[f]	X	X																		
Hepatitis B & C and HIV screens	X																			
Randomization[g]			X																	
AZD6140/placebo administration			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Digoxin administration								X	X	X	X	X	X	X	X	X				
AZD6140 PK samples[h]			X			X	X								X	X				
Digoxin PK samples (blood) [h]			X											X	X	X	X	X	X	
Urine collection for digoxin PK[h]			X													X				
Genetic blood sample[g]			X																	
AE/concomitant medications	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Confinement to clinical research unit		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

Electrocardiogram (ECG), human immunodeficiency virus (HIV), pharmacokinetic (PK), adverse event (AE)

[a]Includes height and weight; performed at screening only

[b]Vital signs include blood pressure and heart rate and will be collected in the morning (pre-dose); body temperature measured on Day -1 (Study Period 1 only)

[c]Laboratory testing includes clinical chemistry, hematology and urinalysis parameters as specified in Section 4.4.7.1 and will be collected pre-dose. PT, aPTT at screening only.

[d]Trough digoxin levels collected approximately 2 hours prior to dosing; test results must be received and reviewed by the investigator prior to administration of digoxin

[e]Required for female subjects only; serum pregnancy test performed at screening and follow-up, urine pregnancy test at Day -1 (Study Periods 1 and 2)

[f]Alcohol breath test required on Day -1 only (Study Periods 1 and 2); urine drugs of abuse screen to be performed at screening and on Day -1 (Study Periods 1 and 2)

[g]Performed pre-dose on Day 1 only (Study Period 1)

[h]Refer to Table 2 for sampling schedule

[i]Liver function tests only; refer to Section 4.4.7.1

[j]Electrolytes only; refer to Section 4.4.7.1

[k]12-lead ECGs performed pre-dose, 2 and 6 hours post-dose (note: AM dose only on Day 6)

[l]Follow-up evaluations performed 7-14 days after Study Period 2

Table 2 Pharmacokinetic Sampling Schedule (Study Periods 1 and 2)

Plasma Sampling		
Study Day	**AZD6140/AR-C124910XX**	**Digoxin**
1	pre-dose	pre-dose
4	pre-dose	--
5	pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours post-dose	--
12	--	pre-dose
13	pre-dose	pre-dose
14	pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48 and 72 hours post-dose	pre-dose, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 36, 48, and 72 hours post-dose
Urine Sampling Intervals		
Study Day	**Digoxin**	
1	pre-dose	
14	0-6, 6-12, and 12-24 hours post-dose	

Note: All pre-dose plasma samples will be taken within 15 minutes prior to dose administration.

3.2 Rationale for study design, doses and control groups

As previously discussed, digoxin is a known P-glycoprotein (Pgp, MDR1) transporter substrate and increased digoxin levels have been observed with concomitant administration of Pgp substrates and/or inhibitors such as verapamil and atorvastatin. It is likely that digoxin will be co-administered with AZD6140 in the intended target population and pre-clinical studies indicate that AZD6140 is a substrate for Pgp. Therefore, the potential interaction between AZD6140 and digoxin will be investigated.

A placebo group is used for comparisons of safety data between the treatment and placebo groups. This study will be a crossover design, which will allow for intra-subject comparison of pharmacokinetic values.

Digoxin will be dosed as a loading dose and then once daily for 8 days. Digoxin has a long half-life, therefore this dosing period is necessary to reach steady state. AZD6140 will be dosed for 5 days to reach steady state, and will be continued throughout the dosing period after the addition of digoxin.

Young healthy subjects will be enrolled to minimize the effects of concomitant disease states or medications on pharmacokinetic parameters.

The objective of this study is to evaluate the effect of repeated doses of AZD6140 on the pharmacokinetics of steady state digoxin. A dose of 400 mg of AZD6140 has been chosen because this is expected to be a clinically relevant dose. The digoxin dose of 0.25 mg per day has been chosen because this is a dose commonly used in clinical practice and is expected to be safely tolerated in healthy subjects.

Digoxin is a narrow therapeutic index drug. Therefore the confidence intervals of 80-125% were selected because small changes in digoxin levels may be clinically significant. The confidence intervals of 70-143% for AZD6140 were selected since doses up to 600 mg per day of AZD6140 have been well tolerated without increases in clinically significant adverse events. Therefore it is anticipated the therapeutic margins for AZD6140 are wider than those for digoxin.

Genetic analysis of the genes that are involved in the disposition of and response to AZD6140 including the potential interaction between AZD6140 and digoxin, such as the MDR-1 and CYP3A genes, will be performed. The $P2Y_{12}$ receptor may also be examined. The genetic data from this study may be pooled with genetic results from other studies on AZD6140 to generate hypotheses to be tested in future studies.

3.3 Selection of study population

3.3.1 Study selection record

The investigator must keep a record of all subjects who were considered for enrollment (signed an informed consent form). Subjects who do not successfully complete screening must not be re-screened without prior approval from the sponsor.

3.3.2 Inclusion criteria

For inclusion in the study, subjects must fulfill all of the following criteria:

1. Provide signed written informed consent;

2. Be healthy male or post-menopausal (cessation of menses for at least 12 months) or surgically sterile female;

3. Be aged 18 to 65 years inclusive;

4. Weigh at least 50 kg and have a body mass index (BMI) between 18 and 30 kg/m^2 inclusive unless approved by the sponsor and investigator;

5. Have normal physical examination, laboratory values, 12-ECG and vital signs, unless the investigator considers an abnormality to be clinically irrelevant;

6. Agree to comply with all requirements of the study (see section 3.3.4).

3.3.3 Exclusion criteria

Any of the following is regarded as a criterion for exclusion from the study:

1. Clinical evidence of Wolff-Parkinson-White syndrome, intermittent complete heart block, second degree heart block or prolonged PR interval of greater than 217 msec on the 12-lead ECG;

2. Resting heart rate less than or equal to 40 beats per minute;

3. History of neurological, hematological, psychiatric, gastrointestinal, hepatic or renal disease or other condition known to interfere with the absorption, distribution, metabolism or excretion of drugs as determined by the investigator;

4. Current and/or past history of intolerance, unexpected response or hypersensitivity to drugs with a similar chemical structure or mechanism of action to AZD6140 or any ingredient in its formulation (refer to section 3.4.1). These drugs include, but are not limited to clopidogrel (Plavix) and ticlopidine (Ticlid);

5. Current and/or past history of intolerance, unexpected response or hypersensitivity to drugs with a similar chemical structure or mechanism of action to digoxin or any ingredient in its formulation

6. Symptoms of any clinically significant illness within 3 weeks prior to Study Period 1, Day 1;

7. Clinically significant out-of-range values for prothrombin time (PT) or activated partial thromboplastin time (aPTT) as judged by the investigator;

8. A personal or family history of bleeding diatheses or a reasonable suspicion of vascular abnormalities including aneurysms;

9. A personal history of severe hemorrhage, hematemesis, melena, hemoptysis, severe epistaxis, menorrhagia or intracranial hemorrhage;

10. History of significant rectal bleeding, as determined by the investigator, within 3 months prior to screening;

11. History of surgery or significant trauma within 3 months prior to randomization unless approved by the sponsor and investigator;

12. Scheduled surgery, including dental surgery, within 2 weeks of completion of the study unless approved by the sponsor and investigator;

13. Blood donation and/or sampling in excess of 450 mL within the previous 12 weeks prior to screening;

14. Consumption of aspirin, ibuprofen or any other drug known to increase the propensity for bleeding within 2 weeks prior to randomization unless approved by the sponsor and investigator;

15. Other than those medications previously discussed, use of any prescribed medication or over-the-counter preparations including herbal preparations, vitamins and nutritional supplements in the 3 weeks prior to randomization unless otherwise approved by the sponsor and investigator. Use of hormone replacement therapy for female subjects is permitted as long as there has been no change in the dosing regimen for at least 3 months prior to screening;

16. Current and/or past history (within 1 year) of alcohol or substance abuse or a positive test for drugs of abuse unless resulting from a declared and confirmed medication and approved by the sponsor and investigator;

17. Use of tobacco or history of use of tobacco or nicotine-containing products in the 3 months prior to randomization unless approved by the sponsor and investigator;

18. Consumption of any products, medicines or nutritional supplements containing Seville oranges or grapefruit within 1 week prior to randomization;

19. Strenuous physical exercise, use of alcohol or caffeine-containing drinks or foods (eg, coffee, tea, cocoa, chocolate and cola) within 48 hours prior to admission to the clinical research unit unless approved by the sponsor and investigator;

20. Positive results of human immunodeficiency virus (HIV) antibody, hepatitis B surface antigen (HBsAg) or hepatitis C antibody testing;

21. Positive results of pregnancy test;

22. Use of any other investigational compound or participation in another clinical trial within 60 days prior to randomization;

23. Employees of the clinical research unit conducting this study;

24. Subjects, who in the opinion of the investigator, should not participate in this study.

3.3.4 Restrictions

3.3.4.1 Dietary Restrictions

All subjects will be served standardized meals during the confinement periods at the clinical research unit, and no other foods will be permitted. The menus will be identical for both Study Period 1 and 2. In addition, the menus will be identical on Day 5 and Day 14 of both Study Periods. Copies of the menus will be provided to the sponsor.

Breakfast will be served 1 hour after the morning dose administration except for Days 5 and 14 (Study Periods 1 and 2). On these study days, the subjects will remain fasting until 4 hours after the morning dose. Water will be allowed ad lib except from 2 hours before until 2 hours after dose administration on Days 5 and 14 (Study Periods 1 and 2).

Subjects will be required to abstain from alcohol and caffeine-containing food or beverages (eg, coffee, tea, cocoa, chocolate, and cola) within 48 hours prior to dose administration and throughout the confinement period(s).

Seville oranges and grapefruit containing products will be restricted for 1 week prior to receiving study medication and throughout the duration of the study.

3.3.4.2 Activity and Concomitant Medication Restrictions

Subjects will be required to:

1. Be confined to the clinical research unit following check-in on Day -1 until discharge on Day 17 for Study Periods 1 and 2;

2. Refrain from strenuous physical exercise from 48 hours prior to receiving study medication until discharge from the study following the final clinical laboratory assessments;

3. Refrain from using tobacco or nicotine containing products throughout the study;

4. Refrain from taking aspirin, ibuprofen or any other drug known to increase the propensity for bleeding from 2 weeks prior to receiving study medication and throughout the study;

5. Refrain from taking over-the-counter medications, including herbal remedies and vitamin preparations, and prescription medications from 3 weeks prior to receiving

study medication and throughout the study, unless approved by the sponsor and investigator;

3.3.5 Discontinuation of subjects from treatment or assessment

3.3.5.1 Criteria for discontinuation

Subjects may be discontinued from study treatment and assessments at any time, at the discretion of the investigator(s). Specific reasons for discontinuing a subject from this study are:

1. Voluntary discontinuation by the subject, who are at any time free to discontinue their participation in the study without prejudice to further treatment.

2. Safety reasons as judged by the investigator or AstraZeneca. In particular, any clinical signs or symptoms of digoxin toxicity as judged by the investigator.

3. Protocol non-compliance, which in the judgment of the sponsor and investigator has the potential to significantly affect the integrity of the data.

3.3.5.2 Procedures for discontinuation

Subjects who discontinue from the study should always be asked about the reason(s) for their discontinuation and about the presence of any adverse events. If possible, they should be seen and assessed by an investigator(s) in addition to completing the exit procedures and evaluations. Adverse events should be followed up and any diary cards, questionnaires and investigational products should be returned by the subject, as appropriate.

The case report forms (CRFs) should be completed to reflect the reason the subject discontinued from the study.

Withdrawal includes having one’s DNA sample destroyed to prevent it from being analyzed. All samples will be destroyed within 10 years. Data generated from genetic analyses will not be destroyed.

If a subject is withdrawn or drops out, he/she will be replaced at the discretion of the sponsor.

3.4 Treatment(s)

3.4.1 Investigational product(s)

3.4.1.1 Identity of investigational product

Table 3 Identity of investigational product

Investigational product	Dosage form and strength	Manufacturer	Formulation number	Ingredients
AZD6140	200 mg immediate release tablet	AstraZeneca	266	AZD6140 Lactose monohydrate Microcrystalline cellulose Polyvinylpyrrolidone K30 Croscarmellose sodium Magnesium stearate Opadry Pink (ref 33G24513)
AZD6140	Placebo to match 200 mg immediate release tablets	AstraZeneca	234	Lactose spray dried Avicel PH102 Magnesium stearate Opadry Pink (ref 33G24513)

Digoxin 0.25 mg tablets will be supplied by the Principal Investigator.

3.4.1.2 Labeling

The supplies will be labeled in accordance with Good Manufacturing Practice (GMP) and will include the following information:

- Sponsor name (AstraZeneca) and address
- Product name
- Dosage form
- Quantity of dosage units
- Study code

- Randomization number
- Study period number
- Directions for use
- Storage conditions
- The following standard statements:
  - "Caution: New Drug – Limited by Federal (United States) Law to Investigational use"
  - "keep out of reach of children"

3.4.1.3 Packaging

Each subject will be assigned a kit according to the randomization schedule. The kit will contain one bottle of AZD6140 tablets and one bottle of AZD6140 matching placebo tablets. Each bottle of AZD6140 or placebo will be labeled with the appropriate study details, including the study period number, according to the randomization schedule. The bottle labels will have a tear-off portion that will be inserted into the CRF.

3.4.1.4 Storage

All investigational products must be kept in a secure place under appropriate storage conditions.

Storage of AZD6140

AZD6140 tablets and placebo must be stored between 15°C and 30°C, protected from light and high humidity. The storage location will be locked and only accessible to authorized study site personnel.

Storage of Digoxin

Digoxin tablets (supplied by the Principal Investigator) should be stored according to the manufacturer's instructions.

3.4.1.5 Accountability

The medication provided for this study is for use only as directed in the protocol. Investigational site personnel or the monitor will return all unused drugs to AstraZeneca. The investigational site personnel will account for all drugs dispensed and returned. Certificates of delivery and return must be signed. The dispensing records must include (at a minimum): the name of the study drug, the subject to whom the study drug was dispensed, the quantity of study drug dispensed and the date and time of dispensing. All study drugs must be accounted

for including any study drug that is lost or destroyed. These records are in addition to dosing information recorded on the subject's CRF.

3.4.2 Doses and treatment regimens

Subjects fulfilling all of the eligibility criteria will be randomized at Day 1, Study Period 1.

Each subject will receive the following treatments, the order as determined by the randomization schedule:

> **Treatment A:** AZD6140 400 mg will be administered once daily in the morning on Days 1-16. A loading dose of digoxin 0.5 mg (0.25 mg bid) will be administered on Day 6 (once in the morning with AZD6140 and again in the evening approximately 12 hours after the morning dose). On Days 7-14 digoxin 0.25 mg will be co-administered with AZD6140 in the morning.
>
> **Treatment B:** AZD6140 placebo will be administered once daily in the morning on Days 1-16. A loading dose of digoxin 0.5 mg (0.25 mg bid) will be administered on Day 6 (once in the morning with AZD6140 placebo and again in the evening approximately 12 hours after the morning dose). On Days 7-14 digoxin 0.25 mg will be co-administered with AZD6140 placebo in the morning.

There will be a minimum 2-week washout period between Study Period 1 and 2.

The investigator or designee will administer all doses of study drug with 240 mL of room temperature water. The subject will be instructed not to crush or chew the tablets. The study drugs will be given when the subject is sitting upright or semi-recumbent. On all study days, the subject must remain upright (standing, sitting or semi-recumbent) for at least 4 hours following dose administration.

On Days 5 and 14 (Study Period 1 and 2), the subject will receive the dose of study drug(s) following an overnight fast of at least 10 hours and will remain fasting until 4 hours post-dose. On the remaining study days, breakfast will be served 1 hour after dose administration. Water will be restricted, other than what is required for dosing, from 2 hours before until 2 hours after dose administration on Days 5 and 14 (Study Period 1 and 2).

3.4.3 Method of assigning subjects to sequences

Written informed consent will be obtained before enrollment and the subjects identified with an enrollment number starting with E0001001 and continuing consecutively. On Day 1 of Study Period 1, subject eligibility will be confirmed prior to sequence randomization. Subjects fulfilling the eligibility criteria will be assigned subject (randomization) numbers starting with number 501. The randomization schedule will be generated in blocks by Quantitative Decision Sciences (QDS) Statistical Programming using the Global Randomization tool (GRand).

Subjects will be randomized strictly sequentially as subjects are eligible for enrollment/randomization. If a subject discontinues from the study, the subject number will not be re-used and the subject will not be allowed to re-enter the study.

Subjects who discontinue prematurely will not be replaced unless it appears that sufficient subjects will not complete the study. The randomization of replacement subjects will be at the discretion of the sponsor.

3.4.4 Blinding and procedures for unblinding the study

3.4.4.1 Methods for ensuring blinding

Since this is a double-blind study, AZD6140 or placebo will be blinded to both the subjects and to the investigator. The study drug will be blinded by providing AZD6140 and matching placebo tablets, which will be indistinguishable in appearance. The digoxin tablets administered to each subject will not be blinded. Packaging, labeling and preparation of investigational products will be performed in a way that will ensure the blinding of AZD6140 throughout the study. Neither the sponsor's representative responsible for monitoring the study, the study personnel, nor the investigator will know whether AZD6140 or placebo has been allocated for each subject.

3.4.4.2 Methods for unblinding the study

Individual treatment codes, indicating the treatment randomization for each randomized subject, will be available to the investigator(s) or pharmacists at the study center.

The individual treatment codes must not be broken except in medical emergencies when the appropriate management of the subject necessitates knowledge of the treatment randomization. The investigator(s) must document and report to AstraZeneca any breaking of the treatment code. AstraZeneca retains the right to break the code in order to report serious adverse events to regulatory authorities.

Treatment codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual subject have been made and documented.

3.4.5 Concomitant medication

Subjects should refrain from taking prescribed or over-the-counter (OTC) medications including herbal remedies, vitamins and nutritional supplements from 3 weeks prior to randomization and throughout the study unless approved by the sponsor and the investigator. Use of hormone replacement therapy for female subjects is permitted as long as the therapy has been stable for at least 3 months prior to screening.

Aspirin, ibuprofen or any other drug known to increase the propensity for bleeding is prohibited from 2 weeks prior to randomization and throughout the study.

During the screening evaluations, each subject will be instructed not to take any medication (prescription or OTC) until discharged from the study without prior approval from the investigator.

Any medication, which is considered necessary for the subject's safety and well-being, may be given at the discretion of the investigator(s). The administration of all medication (including investigational products) must be recorded in the appropriate sections of the case report form (CRF).

3.4.6 Treatment compliance

Compliance will be ensured by supervised administration of the investigational product by the investigator or his/her designee.

4. MEASUREMENT OF STUDY VARIABLES

The following study measurements will be obtained. The timing of these measurements is detailed in the study plan (Table 1). The following 'priority order' will be in effect when more than one assessment is required at a particular timepoint:

- Pharmacokinetic blood sampling will take priority over all study procedures except for study drug administration. Pre-dose samples should be obtained shortly (within 15 minutes) prior to dose administration.

- For logistical purposes, it may be necessary to perform safety assessments (12-lead ECGs, vital signs, safety laboratory tests and adverse event questioning) prior to the protocol timepoint.

4.1 Screening and demographic measurements

Any subject considered for enrollment into the study must provide written informed consent (signed and dated) prior to conducting any study-specific procedures.

4.1.1 Enrollment medical examination

Each subject will undergo an enrollment medical examination in the 21 days prior to randomization. Refer to the study plan (Table 1) for the list of procedures and assessments to be performed at screening. Refer to section 4.4 for a detailed description of these procedures and assessments.

4.1.2 Follow-up medical examination

A follow-up medical examination will be performed 7-14 days after Day 17, Study Period 2. Refer to the study plan (Table 1) for the list of procedures and assessments to be performed. Refer to section 4.4 for a detailed description of these procedures and assessments.

4.2 Pharmacokinetic measurements

For timing of individual samples refer to the study plan (Table 1) and the pharmacokinetic sampling schedule (Table 2).

4.2.1 Collection of biological samples

Samples for the measurement of concentrations of AZD6140 and its metabolite, AR-C124910XX, as well as digoxin will be analyzed using validated bioanalytical methods. Full details of the methods used will be detailed in the clinical study report.

4.2.1.1 Blood samples for determination of AZD6140 and AR-C124910XX

Blood samples (2 mL) for determination of AZD6140 and AR-C124910XX levels in plasma will be taken at the times given in the study plan (Table 1) and pharmacokinetic sampling schedule (Table 2). Blood samples will be collected, labeled and shipped as detailed below. The date and time of collection will be recorded on the appropriate CRF.

Sample collection and handling instructions

After applying a tourniquet, venous blood samples (2 mL) will be drawn from indwelling catheters or by direct venipuncture into collection tubes containing lithium heparin. If indwelling catheters are used, they should be kept patent with isotonic saline; the saline will be withdrawn (1 mL) and discarded before the blood sample is taken. After the sample is collected, the blood and lithium heparin will be mixed carefully and placed in an ice bath until processing begins. Within 30 minutes of collection, the samples will be centrifuged for 10 minutes at 4°C at a relative centrifugal force of 1500 g. The resulting plasma will be transferred to plain polypropylene tubes and immediately frozen upright at -20°C or below in a non-frost free freezer. The samples will be kept frozen at this temperature before, during and after transport to the designated laboratory.

Sample labeling instructions

The sponsor will provide labels for the pharmacokinetic samples. The labels will include the following information:

- Study number (D5130C05265)
- Analyte (AZD6140/AR-C124910XX)
- Randomization/Subject number
- Study period number (Study period 1 or 2)
- Study day (Day 1, 4, 5, 13 or 14)
- Scheduled/Protocol time

The label must only be used for the intended sample and the pre-printed information must not be changed.

Sample shipment instructions

All pharmacokinetic plasma samples will be shipped via an agreed upon overnight courier. Plasma samples will be shipped to the address below. The samples must be packed securely to avoid breakage during transit, double-bagged to contain leaks and packed with a sufficient quantity of dry ice to ensure they remain frozen for at least 72 hours. All applicable shipping regulations must be followed. Documentation sufficient to identify each sample must be included in the shipment. The primary contact at AstraZeneca, Patty Davis, and the designated laboratory identified below must be notified by telephone, fax or email **before the samples are shipped**.

Ship samples on a Monday, Tuesday or Wednesday. Do not ship on or the day before a local holiday.

AZD6140/AR-C124910XX samples along with the corresponding documentation will be shipped to:

Notification to DMPK Wilmington:

4.2.1.2 Blood samples for determination of digoxin

Blood samples (2 mL) for determination of digoxin levels in plasma will be taken at the times given in the study plan (Table 1) and pharmacokinetic sampling schedule (Table 2). Blood samples will be collected, labeled and shipped as detailed below. The date and time of collection will be recorded on the appropriate CRF.

Sample collection and handling instructions

After applying a tourniquet, venous blood samples (2 mL) will be drawn from indwelling catheters or by direct venipuncture into collection tubes containing sodium heparin. If indwelling catheters are used, they should be kept patent with isotonic saline; the saline will be withdrawn (1 mL) and discarded before the blood sample is taken. After the sample is collected, the blood and sodium heparin will be mixed carefully and placed in an ice bath until processing begins. Within 30 minutes of collection, the samples will be centrifuged for 10 minutes at 4°C at a relative centrifugal force of 1500 g. The resulting plasma will be transferred to plain polypropylene tubes and immediately frozen upright at - 80°C or below in a non-frost free freezer. The samples will be kept frozen at this temperature before, during and after transport to the designated laboratory.

Sample labeling instructions

The sponsor will provide labels for the pharmacokinetic samples. The labels will include the following information:

- Study number (D5130C05265)
- Analyte (digoxin)
- Randomization number
- Study period number (Study period 1 or 2)
- Study day
- Scheduled/Protocol time

The label must only be used for the intended sample and the pre-printed information must not be changed.

Sample shipment instructions

All pharmacokinetic plasma samples will be shipped via an agreed upon overnight courier. Plasma samples will be shipped to the address below. The samples must be packed securely to avoid breakage during transit, double-bagged to contain leaks and packed with a sufficient quantity of dry ice to ensure they remain frozen for at least 72 hours. All applicable shipping regulations must be followed. Documentation sufficient to identify each sample must be included in the shipment. The primary contact at AstraZeneca, Patty Davis, and the designated laboratory identified below must be notified by telephone, fax or email **before the samples are shipped**.

Ship samples on a Monday, Tuesday or Wednesday. Do not ship on or the day before a local holiday.

Digoxin samples along with the corresponding documentation will be shipped to:

Notification to DMPK Wilmington:

4.2.1.3 Collection of urine for determination of digoxin

Each subject will pass all urine into a specially provided container(s) at the times given in the study plan (Table 1) and pharmacokinetic sampling schedule (Table 2). Samples will be collected, labeled and shipped as detailed below. The date and the time of collections will be recorded on the appropriate CRF.

Sampling collection and handling instructions

A urine sample of at least 10 mL will be collected at the timepoints specified in Table 2.

Just prior to the start of the first urine collection interval, the subject will empty his/her bladder. This time will be recorded as the start of the first collection interval. Within approximately 15 minutes before the end of each collection interval, the subject will be asked to void. This will be recorded as the end time of the interval, if the subject is able to void. During each of the urine collection intervals, the urine will be stored at ~4°C. At the end of the collection interval, the urine pool will be thoroughly mixed by inverting the container at least 10 times. The total weight of the urine collected will be measured and recorded. One 10 mL aliquot of the well-mixed urine pool from each collection interval will be transferred into labeled 15.0 ml polypropylene tubes (In addition, a sample from each urine pool will be analyzed by the clinical research unit for urinary creatinine concentration according to standard practice.) The urine aliquots including the pre-dose sample will be stored in an upright position at or below -80°C in a non-frost free freezer). The samples will be kept frozen at this temperature before, during and after transport to the designated laboratory.

Sample labeling instructions

The sponsor will provide labels for the urine samples. The labels will include the following information:

- Study number (D5130C05265)
- Randomization number
- Study period number (Study period 1 or 2)
- Study day
- Scheduled/Protocol time or interval

The label must only be used for the intended sample and the pre-printed information must not be changed.

Sample shipment instructions

All urine samples will be shipped via an agreed upon overnight courier. The samples will be shipped to the address below. The samples must be packed securely to avoid breakage during transit, double-bagged to contain leaks and packed with a sufficient quantity of dry ice to ensure they remain frozen for at least 72 hours. All applicable shipping regulations must be followed. Documentation sufficient to identify each sample must be included in the shipment. The primary contact at AstraZeneca, Patty Davis, and the designated laboratory identified below must be notified by telephone, fax or email **before the samples are shipped**.

Ship samples on a Monday, Tuesday or Wednesday. Do not ship on or the day before a local holiday.

Urine samples along with the corresponding documentation will be shipped to:

Notification to DMPK Wilmington:

4.3 Pharmacodynamic measurements (Not applicable)

4.4 Screening and safety measurements

For timing of individual assessments and procedures, refer to the study plan (Table 1).

Information collected for screen failures will be limited to demographic information and the reason for failure of screening including the entrance criteria that were not met and the reason for discontinuation.

4.4.1 Demographics

Demographic information will be collected for all subjects who are enrolled. These data will include gender, date of birth, race, and date of informed consent.

4.4.2 Review of inclusion/exclusion criteria

The inclusion and exclusion criteria must be assessed and reviewed with each subject in order to establish and confirm eligibility to participate or continue in the study.

4.4.3 Medical/surgical history

A detailed medical and surgical history will be recorded for each subject. Significant medical conditions and surgical events will be recorded on the appropriate CRF.

4.4.4 Medication history

The medication history must identify any known drug allergies, presence or history of drug or alcohol abuse. All prescribed medications and OTC products (including herbal remedies, vitamins or nutritional supplements) taken within 3 weeks prior to randomization will be recorded on the appropriate CRF. During the screening evaluations, each subject will be instructed not to take any medication (prescription or OTC) until discharged from the study without prior approval from the investigator.

4.4.5 Complete physical examination

The complete physical examination will include an assessment of the following: general appearance, skin, head and neck (including eyes, ears, nose and throat), lymph nodes, thyroid, musculoskeletal/extremities (including spine), cardiovascular, lungs, abdomen and neurological (reflexes). Height (cm) and weight (kg) will be measured without shoes and BMI will be calculated. Height and weight will be measured and BMI calculated at screening only.

4.4.6 Brief physical examination

The brief physical examination will include an assessment of the following: general appearance, abdomen, cardiovascular, lungs and other (if applicable). If the subject states changes have occurred related to systems not assessed, then these systems should also be examined and the data recorded on the CRF. If there has been no change from the previous examination, only that information will be recorded.

4.4.7 Laboratory safety measurements

4.4.7.1 Clinical chemistry, hematology, coagulation, and urinalysis

Blood and urine samples for determination of clinical chemistry, hematology, coagulation and urinalysis parameters will be taken at the times given in the study plan (Table 1). The date and time of collection will be recorded on the appropriate CRF.

Samples will be collected according to standard practice at the clinical research unit.

The following laboratory variables will be measured:

Clinical chemistry

Glucose (fasting)
Sodium
Potassium
Calcium
Chloride
Magnesium
Albumin
Total bilirubin
Alkaline phosphatase
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Gamma glutamyltransferase (GGT)
Blood urea nitrogen
Creatinine
Total protein
Uric Acid

Hematology

Hemoglobin
Hematocrit
Platelet count
Red blood cell (RBC) count
White blood cell (WBC) count and differential (%) including: neutrophils, lymphocytes, monocytes, eosinophils, basophils

Coagulation (Screening only)

Prothrombin time (PT)
Activated partial thromboplastin time (aPTT)

Urinalysis

Protein
Glucose
Blood
Microscopy (if positive for blood or protein)

Limited chemistry evaluations will be measured as follows on Study Period 1 and 2:

- Day 2 and 7 (pre-dose): Total bilirubin, ALT, AST, GGT and alkaline phosphatase
- Day 7 (pre-dose): Sodium, potassium, calcium, chloride, and magnesium

On Day 14 of Study Period 1 and 2, urinary creatinine will be measured at the end of the collection interval for each of the well-mixed urine pools.

4.4.7.2 Stat digoxin levels

Trough serum blood samples will be taken at the times specified in the study plan (Table 1) for subject safety. Samples will be collected approximately 2 hours prior to dosing according to standard practice at the clinical research unit. The test results must be received from the laboratory and reviewed by the investigator prior to the subsequent dose of digoxin. The date and time of collection will be recorded on the appropriate CRF.

4.4.7.3 Drugs of abuse screen

Urine will be tested for the following drugs of abuse:

Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates

Alcohol breath tests will be conducted on check-in to the clinical research unit at Study Periods 1 and 2.

The test results will be confirmed negative prior to randomization or continuation in the study, but will not be entered into the database.

If a subject tests positive for any drugs of abuse, the subject will be excluded from entering, or continuing in, the study.

4.4.7.4 Virology screen

A blood sample to screen for human immunodeficiency virus (HIV) antibody, hepatitis B surface antigen (HBsAg) and hepatitis C antibody will be obtained. The test results will be confirmed negative prior to randomization, but will not be entered into the database. If a test result is positive the subject will be excluded from entering the study.

4.4.7.5 Pregnancy test

A serum pregnancy test will be performed on all female subjects at the screening and follow-up visits. Urine pregnancy tests will be performed on check-in to the clinical research unit for Study Period 1 and 2. The test results will be confirmed negative prior to randomization or continuation in the study, but will not be entered into the database.

4.4.8 Electrocardiographic measurements

For timing of individual measurements refer to study plan (Table 1).

4.4.8.1 Resting 12-lead ECG

Twelve-lead ECGs will be obtained after the subject has been lying down for 10 minutes in each case. All ECGs will be evaluated as normal or abnormal by the investigator. Additional ECG assessments may be obtained at the discretion of the investigator if a condition develops that warrants additional monitoring.

4.4.9 Vital signs

4.4.9.1 Blood pressure and heart rate

For timing of individual measurements refer to study plan (Table 1).

In the morning on each study day (pre-dose), blood pressure and heart rate (HR) will be measured after the subject has been sitting for at least 5 minutes. Body temperature will be measured at Day -1 (Study Period 1 only).

4.5 Genetic sampling and storage

A single 9 mL venous blood sample will be collected into a polypropylene EDTA tube pre-dose on Day 1 of Study Period 1 only for use in genetic testing. This sample will be

optional and the subject may choose to participate in the study but not to provide this blood sample for genetic analysis. A separate consent will be required for genetic sampling.

The sample will be frozen as whole blood at -20°C or below in a non-frost free freezer and transported to the assaying laboratory within one month of collection. Processing, labeling and shipping instructions are provided in Appendix D.

Genetic samples will be sent to the following address:

4.6 Volume of blood sampling

The total volume of blood that will be drawn from each subject in this study is as follows:

Table 4 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	n of samples	Total volume (mL)
PK samples for AZD6140/metabolite		2	60	120
PK samples for digoxin		2	38	76
Genetic sample		9	1	9
Safety	Clinical chemistry	10	10	100
	Hematology	5	6	30
	Coagulation	4.5	1	4.5
	Digoxin levels	5	9	45
	Serum pregnancy	1	2	2
	Virology	10	1	10
Total				**396.5**

4.7 Adverse Events

The definitions of adverse events (AEs), serious adverse events (SAEs) and other significant adverse events (OAEs) and the recording of AEs are given in Appendix B. It is of the utmost

importance that all staff involved in the study are familiar with the content of these sections. The principal investigator is responsible for ensuring this occurs.

4.7.1 Recording of adverse events

The subjects will be told to report any AE occurring during the study to the investigator or his/her personnel. Open, standardized AE questioning such as, "Has anything bothered you since you were asked last?", will be done by the investigator(s) or his/her personnel at each contact with the subject. The AE open, standardized questioning should be done discretely in order to prevent the subjects from influencing each other.

Information about AEs will be collected from the first administration of study drug until the completion of the follow-up visit. Serious adverse event information will be collected from the time the subject signs the informed consent until 14 days following the last dose of study drug at Study Period 2. Any AEs observed or reported by a subject and/or staff will be recorded in the CRF. Any AE including clinical findings not resolved on the final dosing day will be followed until resolved or explained.

Laboratory and vital signs abnormalities will not be recorded as an AE unless any criterion for an SAE is fulfilled, the subject discontinues the study due to the result(s), or the investigator considers it to be of such clinical importance as to merit recording it as an AE. If a laboratory value or vital sign is associated with clinical signs or symptoms, the signs or symptoms should be reported as an AE and the associated laboratory value or vital sign should be considered additional information. Any sign or symptom that fulfills SAE definition (Appendix B) or are the reason for discontinuation of study drug should be reported accordingly (refer to section 4.7.2).

To avoid colloquial expressions, the AE term should be reported in standard medical terminology when possible. For each AE, the investigator will evaluate and report the onset (date and time), resolution (date and time), intensity, causality, action taken, serious outcome (if applicable) and whether or not it caused the subject to discontinue the study.

The following variables will be recorded for each AE:

Onset, resolution, maximum intensity, action taken, outcome, causality (yes or no) and whether it constitutes an SAE or not.

The intensity rating is defined as:

1 = mild (awareness of sign or symptom, but easily tolerated)

2 = moderate (discomfort sufficient to cause interference with normal activities)

3 = severe (incapacitating, with inability to perform normal activities)

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Appendix B. An AE of severe

intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

AEs will be classified using the most current version of the Medical Dictionary for Regulatory Activities (MedDRA). Coding of AEs will be made in the Astra Monitoring System (AMOS).

4.7.2 Reporting of serious adverse events

When the investigator becomes aware of an SAE during the course of the study, the SAE must be reported to the local monitor or other AstraZeneca representative within one (1) day.

All SAEs must be reported, whether or not considered causally related to the investigational product. All SAEs will be recorded in the case report form. The investigator is responsible for informing the Ethics Committee and/or the Regulatory Authority of the SAE as per local requirements.

The AstraZeneca representative will work with the investigator to compile all the necessary information to ensure that the appropriate AstraZeneca Drug Safety Department receives a report within one day for all fatal and life-threatening cases and within five days for all other SAEs. Follow-up information on SAEs should also be reported by the investigator in the same time frames. If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca within 1 day as described above.

YOU MUST REPORT ANY SERIOUS ADVERSE EVENT, INCLUDING DEATH DUE TO ANY CAUSE, IMMEDIATELY. COMPLETE THE ASTRAZENECA SERIOUS ADVERSE EVENT REPORT FORM AND CONTACT ONE OF THE PEOPLE LISTED IN THE ASTRAZENECA EMERGENCY CONTACT PROCEDURE ON PAGE 2 OF THIS PROTOCOL.

YOU MUST FOLLOW ALL SUBJECTS WITH A SERIOUS ADVERSE EVENT, INCLUDING DISCONTINUED SUBJECTS, UNTIL RESOLUTION OF THE ADVERSE EVENT.

5. STUDY MANAGEMENT

5.1 Monitoring

5.1.1 Study monitoring

The monitoring of this study will be performed in accordance with the principles of Good Clinical Practice (GCP) as laid out in the International Conference on Harmonisation (ICH) document “Good Clinical Practice: Consolidated Guideline”.

5.1.2 Data verification

It is a prerequisite of this study that the study monitor has direct access to source data for data verification. This will be done by comparing data from the CRFs with those in the subject's medical notes (permission from the subject will be sought as part of the consent process). Such verification is an essential element of quality control, as it allows the rectification of transcription errors and omissions.

Monitoring will routinely be performed prior to the transfer of data to Data Management.

5.2 Archiving of study documentation

AstraZeneca will retain all documentation pertaining to this study in accordance with ICH Guidelines and AstraZeneca company policy.

The investigator will retain all documentation pertaining to this study for at least 15 years.

5.3 Audits and inspections

Authorized representatives of AstraZeneca, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the center to perform audits or inspections, including source data verification. The purpose of an AstraZeneca audit or inspection is to systematically and independently examine all study related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH and any applicable regulatory requirements. The investigator should contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at his or her center.

5.4 Training of staff

The principal investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff). He or she will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

5.5 Changes to the protocol

Study procedures will not be changed without the mutual agreement of the principal investigator and AstraZeneca.

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol must be notified to or approved by each IEC or IRB, and in many countries also the local regulatory authority, before implementation. Local requirements must be followed.

If a protocol amendment requires a change to a particular center's Written Informed Consent Form, then AstraZeneca and the center's IEC or IRB must be notified. Approval of the

revised Written Informed Consent Form by AstraZeneca and by the IEC or IRB is required before the revised form is used.

AstraZeneca will distribute amendments and new versions of the protocol to each principal investigator who in turn is responsible for the distribution of these documents to his or her IEC or IRB, and to the staff at his or her center. The distribution of these documents to the regulatory authority will be handled according to local practice.

5.6 Study agreements

The principal investigator at each center must comply with all the terms, conditions, and obligations of the study agreement for this study. In the event of any inconsistency between this protocol and the study agreement, this protocol shall prevail.

5.7 Study timetable and termination

The study is expected to start in September 2003 and to be completed by December 2003.

Completion of the study

Upon completion of the study, the investigator will inform the IEC or IRB and AstraZeneca in writing that the study is complete, and will include a summary of results.

5.8 Data management

5.8.1 Case report forms

CRFs will be used to record all data not captured electronically. The CRFs will be in triplicate with carbonless paper. Data should be recorded directly and legibly from the source documents onto the CRFs, preferably in black ballpoint pen. Corrections to the CRFs should be made legibly, initialed and dated. Correction fluid or covering labels must not be used. The top original (white) and the first copy (yellow) of each completed CRF will be collected and returned to the AstraZeneca designee; the investigator will retain the second copy (manila).

The AstraZeneca Monitor will check data at the monitoring visits to the investigational site. The Investigator, together with the AstraZeneca Monitor, will ensure that the data in the CRFs are accurate, complete and legible.

AstraZeneca Data Management will enter the CRF data on an ongoing basis into their standard commercial database. The data will be verified and cleaned with electronic edit checks comprised of validated computer programs and manual data review. Any missing, inconsistent or illegible entries into the CRFs will be referred back to the investigator via the site monitor using Data Query Forms within an agreed number of days upon entering the data. Responses should be received by Data Management and updated within an agreed number of days upon generating the data queries. Clean file will be declared when all of the following have been completed: all data have been accounted for and have been databased; all edit checks have been run and data discrepancies have been resolved or accepted; all serious adverse events have been reconciled with the clinical database; all coding is complete and has

been medically reviewed and approved; and quality control of the database against the CRFs and relevant data sources has been completed.

5.8.2 Immediate Data Entry (Not applicable)

5.8.3 Electronic Data Capture (Not applicable)

5.8.4 Pharmacogenetic data

The genotype data will be used to explore genetic factors in the disposition of and response to AZD6140. The results of the genetic study will not form part of the clinical study database or the clinical study report. The results of this study may be pooled with genetic results from other studies on AZD6140 to generate hypotheses to be tested in future studies.

The DNA sample will not be labeled with the subject's name but will only be identified by a code that will link it to other information collected about the study drug during the course of this study. This link will exist to provide a way for regulatory authorities to track and ensure clinical genetic research is being conducted correctly and will be maintained only for the period of time that these authorities require; it will be destroyed after 15 years. If the subject changes his/her mind about this testing after donating a blood sample, this link will be used to track and destroy the sample. The study sponsor can only guarantee the possibility of destroying the sample while the link is maintained.

Test results will be kept confidential in accordance with all applicable laws. None of the test results will be provided to the subject, any insurance company, the subject's employer or family, the investigator or any other physician who is treating the subject or may treat the subject in the future.

The subject's DNA will not be used for any purpose other than genotyping. DNA will not be passed on to any other party and all samples will be retained for a 15-year period.

6. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

6.1 Statistical evaluation

Statistical analysis will be carried out by Biostatistics at AstraZeneca, Wilmington, Delaware using SAS (version 8). Summary graphics required for presentation in the text portion of the Clinical Study Report (CSR) will be done using Sigmaplot 2000. Other graphics intended for use as supplemental figures and individual profile figures will be done using SAS. A comprehensive Statistical Analysis Plan (SAP) will be prepared and finalized before unblinding and analysis of the data.

The goal of this analysis is to compare the pharmacokinetic (PK) parameters $C_{ss,max}$, $C_{ss,min}$, and AUC_{τ} of digoxin in the presence and absence of AZD6140.

6.2 Description of outcome variables in relation to hypotheses

6.2.1 Demographic and baseline data

All demographic variables, including medical and surgical history, physical examination and medications taken before and during the study, as detailed in section 4.1, will be obtained from the CRF.

6.2.2 Pharmacokinetics

Plasma concentrations of digoxin, and AZD6140 and its metabolite AR-C214910XX, will be listed and depicted graphically as a function of time.

The following pharmacokinetic parameters will be calculated at steady state:

- Digoxin: $C_{ss,max}$, t_{max}, $C_{ss,min}$, $C_{ss,av}$, AUC_{τ}, t½, CL/F, and Ae_{24h} (total amount unchanged drug excreted in urine during last dosing interval)
- AZD6140: $C_{ss,max}$, t_{max}, $C_{ss,min}$, $C_{ss,av}$, AUC_{τ}, and CL/F
- AR-C124910XX: $C_{ss,max}$, t_{max}, $C_{ss,min}$, $C_{ss,av}$, and AUC_{τ}

All pharmacokinetic parameters will be will be estimated by non-compartmental analysis.

The $C_{ss,max}$ will be estimated as the highest measured (i.e. observed) plasma concentration over the dosing interval, and the t_{max} will be the time to maximum plasma concentration. $C_{ss,av}$, the average concentration in a dosing interval, will be estimated as the ratio of AUC_{τ} and the dosing interval 'τ' (24 hours). The $C_{ss,min}$ will be estimated as the lowest measured plasma concentration over the dosing interval. The attainment of steady state for AZD6140 and AR-C124910XX will be evaluated from the $C_{ss,min}$ values on Days 4-5 and 13-14, and the corresponding steady state levels of digoxin will be evaluated from $C_{ss,min}$ values on Days 12-14. The primary analysis of digoxin $C_{ss,min}$ described in Section 6.4 will be performed on the data from Day 14 of the study periods.

The AUC_{τ} will be calculated using the linear trapezoidal method during one dosing interval (0-24 h) at steady state on Day 5 (AZD6140 and AR-C124910XX only) and Day 14 (all compounds). AZD6140 CL/F will be estimated as the ratio of AZD6140 dose and AUC.

The terminal elimination rate constant (λ_z) after the last dose of digoxin will be calculated by log-linear regression of the terminal portion of the concentration-time profile. The terminal elimination half-life ($t_{½}$) of digoxin will be calculated as $0.693/\lambda_z$.

The total amount of digoxin excreted in urine during the last dosing interval (Ae_{24h}) will be calculated by adding the total amount digoxin excreted during the collection intervals (digoxin urine concentration x urinary volume).

For digoxin, the pharmacokinetic parameters will be summarized by study period (digoxin + AZD6140 or digoxin + placebo). For AZD6140 and its metabolite, the pharmacokinetic parameters will be summarized within one study period (on Days 5 and 14, respectively).

6.2.3 Safety

The incidence, nature and severity of adverse events will be taken directly from the CRF data. All variables relating to laboratory measurements, ECGs, and vital signs, as detailed in section 4.4 will be included in the analysis. Changes from baseline will be relative to the latest pre-dose value on Day -1 at Study Period 1 and 2.

6.3 Description of analysis sets

Subjects will be included in the primary analysis set for the pharmacokinetic variable in question if they complete both periods of the study with no major protocol violations and they provide adequate data in support of the analyses described below.

If the number of evaluable subjects falls below 12 due to dropouts, then subjects may be replaced in the same sequence as those subjects who dropped.

Subjects will be included in the analysis of safety if they have received at least one dose of study medication.

6.4 Methods of statistical analyses

6.4.1 Demographic and baseline data

All demographic data including medical and surgical history, physical examination and medications taken before and during the study will be summarized and listed using appropriate summary statistics. Data will be summarized by treatment (concomitant medications only). Medications will be coded using WHO Drug Dictionary categories.

6.4.2 Pharmacokinetics

Digoxin $C_{ss,max}$, $C_{ss,min}$, and AUC_{τ} will be the primary endpoints for this study. Primary PK parameters will be log-transformed prior to analysis. The primary model for this study will be based on an analysis of variance (ANOVA) model including terms for treatment (AZD6140 + digoxin, placebo + digoxin), sequence, period and subject within sequence as a random effect. The primary contrast of each of the variables will be AZD6140 + digoxin/placebo + digoxin. The estimates of the primary contrast will be presented using 90% confidence intervals, glsmeans, and p-values.

An absence of a clinically significant effect of AZD6140 on the pharmacokinetics of digoxin will be concluded if the 90% confidence intervals for the ratio of geometric means of the primary parameters ($C_{ss,max}$ and AUC_{τ}) of digoxin are within 80-125%.

While $C_{ss,max}$, AUC_{τ} and CL/F for AZD6140 and $C_{ss,max}$, AUC_{τ} for AR-C124910XX and $C_{ss,min}$ for digoxin are secondary endpoints they will be analyzed in a manner similar to the primary endpoints but pre-specified limits will not be assigned.

An absence of a clinically significant effect of digoxin on the pharmacokinetics of AZD6140 will be concluded if the 90% confidence intervals for the ratio of geometric means of the primary parameters ($C_{ss,max}$ and AUC_{τ}) of AZD6140 are within 70-143%.

In addition, summary statistics will be presented separately for all pharmacokinetic parameters for digoxin by treatment, AZD6140 and AR-C12490XX (before and during digoxin administration).

6.4.3 Safety

6.4.3.1 Adverse events

All adverse events will be listed for each subject including the assigned system organ class (SOC) and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA). All adverse events occurring following the first dose of study drug will be summarized by SOC, preferred term and treatment. The time window for adverse events within a particular treatment will begin at the time of first dose for that treatment up until the time of first dose of the following treatment, or the end of the study. Adverse events will be assigned to the visit in which they first started. Adverse events reported before the first dose of study drug will be listed only.

6.4.3.2 Laboratory and vital sign data

Hematology, clinical chemistry and vital sign data will be listed and summarized by treatment and measurement time. Changes from baseline in each of these parameters will also be summarized using descriptive statistics. Baseline will be defined as the Day -1 measurement for Study Periods 1 and 2. Values that are out of the laboratory reference range will be flagged in the listings. Qualitative urinalysis data will be summarized using the number of subjects with results of negative, trace or positive.

6.4.3.3 ECG data

The ECG data will be listed and summarized by treatment and measurement time.

6.5 Determination of sample size

Twelve (12) evaluable subjects will provide more than a 95% probability that a 2-sided 90% confidence interval for the ratio (AZD6140+digoxin/placebo+digoxin) of C_{max} and $AUC_{(0-24)}$ will be completely contained in the pre-specified limits (0.8-1.25) when the true ratio between treatments is 1 assuming CV's of 15.1 for C_{max} and 11.2 for $AUC_{(0-24)}$ [log-scale SD's of 0.1505 and 0.1112 respectively (Ref. 1)] and an intra-subject correlation of 0.5.

6.6 Interim analyses (Not applicable)

6.7 Data Presentation (Not applicable)

6.8 Data or safety monitoring committee (Not applicable)

7. ETHICS

7.1 Ethics review

The final study protocol and the final version of the Written Informed Consent Form must be approved or given a favorable opinion in writing by the IEC or IRB as appropriate. The investigator must submit written approval to AstraZeneca before he or she can enroll any subject into the study.

The principal investigator is responsible for informing the IEC or IRB of any amendment to the protocol in accordance with local requirements. In addition, the IEC or IRB must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IEC or IRB annually, as local regulations require.

Either the principal investigator(s) or AstraZeneca must submit progress reports to the IEC or IRB according to local regulations and guidelines. The principal investigator(s) must also provide the IEC or IRB with any reports of serious adverse events from the study site.

Under no circumstances will the investigation be extended beyond the limitations defined in this protocol or any subsequent amendments.

7.2 Ethical conduct of the study

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki and are consistent with Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on bioethics.

7.3 Subject information and consent

The principal investigator at each center will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject’s signed and dated informed consent must be obtained before conducting any procedure specifically for the study.

The principal investigator must store the original, signed Written Informed Consent Form. A copy of the Written Informed Consent Form must be given to the subject. If modifications are made according to local requirements, the new version has to be approved by AstraZeneca.

AstraZeneca recognizes the importance of protecting the privacy of subject data. Therefore, for study sites within the United States or in studies where foreign subjects' protected health information (subject data) will come into the United States through a covered entity (eg, Central Lab/Reader), the informed consent form will incorporate, or be accompanied by, a separate document incorporating HIPAA-compliant wording by which subjects authorize the use and disclosure of their protected health information by the investigator and by those persons who need that information for the purposes of the study.

7.4 Subject data protection

The Written Informed Consent Form will explain that study data will be stored in a computer database, maintaining confidentiality in accordance with national data legislation. All data computer processed by AstraZeneca will be identified by subject number/study code. The Written Informed Consent Form will also explain that for data verification purposes, authorized representatives of AstraZeneca, a regulatory authority, an IEC or IRB may require direct access to parts of the hospital or practice records relevant to the study, including subjects' medical history.

8. EMERGENCY PROCEDURES

8.1 Medical emergency contact procedure

In the case of a medical emergency, contact AstraZeneca personnel shown below.

Refer to page 2 for contact information

-
-
-

For Serious Adverse event reporting

- Drug Safety Fax +1 302 886 4114

8.2 Procedures in case of medical emergency

The principal investigator(s) is responsible for ensuring that procedures and expertise are available to cope with medical emergencies during the study.

A medical emergency may constitute a serious adverse event and information on this should be made available to the Drug Safety Physician (Dr. Scott Allender – see page 2) as soon as possible. Refer to section 4.7.2 for further information on serious adverse events and section 3.4.4.2 for unblinding procedures.

In addition, the Clinical Study Team Physician (Kathleen Butler – see page 2) and the Clinical Study Team Leader (Annette Stevenson – see page 2) should be kept informed of any medical emergencies.

8.3 Procedures in case of overdose

There is limited previous human experience regarding the use of AZD6140. In case of overdose, monitoring of cardiac, hepatic and hematological effects is essential and standard supportive therapy should be initiated where appropriate. Since there is no specific antidote to the novel compound, the subject should be treated symptomatically. For further information, see the Clinical Investigator's Brochure.

The subjects will be monitored for signs and symptoms of digoxin toxicity. Any toxicity will be treated symptomatically. Digibind will be available in the clinical research unit, if required.

8.4 Procedures in case of pregnancy

Although females of non-child bearing potential will be selected for this study, in the most unlikely event that pregnancy occurs, the following details will be relevant.

Pregnancy itself is not regarded as an adverse event unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even if the subject was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. All outcomes of pregnancy must be reported to AstraZeneca on the pregnancy outcomes report form.

9. REFERENCE

1. Peeters PA, Crijns HJ, Tamminga WJ, Jonkman JH, Dickinson JP, Necciari J. Clopidogrel, a novel antiplatelet agent, and digoxin: Absence of pharmacodynamic and pharmacokinetic interaction. *Seminars in Thrombosis and Hemostasis* 1999;25(Suppl 2):51-54.

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix A	
Drug substance:	AZD6140
Study Code:	D5130C05265
Appendix Edition No:	Final version 1.0
Appendix Date:	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A Randomized, Double-Blind, Two-Period Crossover Study to Assess Safety, Tolerability, and Pharmacokinetics Following Repeated Doses of AZD6140 (400 mg od) and Digoxin (0.25 mg od) in Healthy Male and Female Volunteers

I agree to the terms of this study protocol

AstraZeneca Clinical Development Team representative

Date
(Day Month Year)

ASTRAZENECA SIGNATURE(S)

A Randomized, Double-Blind, Two-Period Crossover Study to Assess Safety, Tolerability, and Pharmacokinetics Following Repeated Doses of AZD6140 (400 mg od) and Digoxin (0.25 mg od) in Healthy Male and Female Volunteers

I agree to the terms of this study protocol

AstraZeneca Research and Development site representative

Date
(Day Month Year)

ASTRAZENECA SIGNATURE(S)

A Randomized, Double-Blind, Two-Period Crossover Study to Assess Safety, Tolerability, and Pharmacokinetics Following Repeated Doses of AZD6140 (400 mg od) and Digoxin (0.25 mg od) in Healthy Male and Female Volunteers

I agree to the terms of this study protocol

AstraZeneca Research and Development site representative

Date
(Day Month Year)

STUDY NO.: D5130C05265
CENTER NO.: 001
DOC. CODE: 10
DATE TO IM. SRV.:

SIGNATURE OF PRINCIPAL INVESTIGATOR

A Randomized, Double-Blind, Two-Period Crossover Study to Assess Safety, Tolerability, and Pharmacokinetics Following Repeated Doses of AZD6140 (400 mg od) and Digoxin (0.25 mg od) in Healthy Male and Female Volunteers

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and local regulations.

Centre No.: 001

Signature:

Date
(Day Month Year)

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix B	
Drug substance:	AZD6140
Study Code:	D5130C05265
Appendix Edition No:	Final Version 1.0
Appendix Date:	

Appendix B
Additional Safety Information

1. DEFINITIONS OF ADVERSE EVENTS

Adverse event

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

Serious adverse event

A serious adverse event is an AE occurring during any study phase (eg, run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfils one or more of the following criteria:

- results in death
- is immediately life-threatening
- requires in-subject hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital abnormality or birth defect
- is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above?

The causality of SAEs (eg, their relationship to study treatment) will be assessed by the investigator(s), who in completing the relevant case report form must answer “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by the drug?” Further guidance on the definition of a SAE and a guide to the interpretation of the causality question is provided in this Appendix to the Clinical Pharmacology Study Protocol.

Other significant adverse event (OAE)

OAEs will be identified by the Drug Safety Physician and if applicable also by the Clinical Study Team Physician during the evaluation of safety data for the Clinical Study Report. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment, will be classified as OAEs. Examples of these are marked hematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment. For each OAE, a narrative may be written and included in

the Clinical Study Report.

2. FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

Life threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Out-patient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Examples of such events are:

- Angioedema not severe enough to require intubation but requiring iv. hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc.) or convulsions that do not result in hospitalisation
- Development of drug dependency or drug abuse

3. A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?

- Dechallenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Rechallenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a rechallenge.

- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

A "reasonable possibility" could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?

- Is there a known mechanism

Ambiguous cases should be considered as being a "reasonable possibility" of a causal relationship unless further evidence becomes available to refute this. Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

Clinical Pharmacology Study Protocol: Appendix C	
Drug substance:	AZD6140
Study Code:	D5130C05265
Appendix Edition No:	Final Version 1.0
Appendix Date:	

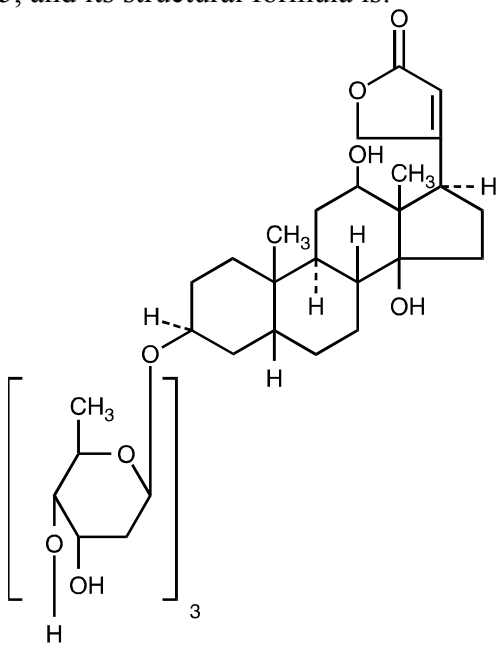
Appendix C
Digoxin Summary of Product Characteristics

PRODUCT INFORMATION

LANOXIN® (digoxin) Tablets, USP
125 mcg (0.125 mg) Scored I.D. Imprint Y3B (yellow)
250 mcg (0.25 mg) Scored I.D. Imprint X3A (white)

DESCRIPTION: LANOXIN (digoxin) is one of the cardiac (or digitalis) glycosides, a closely related group of drugs having in common specific effects on the myocardium. These drugs are found in a number of plants. Digoxin is extracted from the leaves of *Digitalis lanata*. The term "digitalis" is used to designate the whole group of glycosides. The glycosides are composed of two portions: a sugar and a cardenolide (hence "glycosides").

Digoxin is described chemically as (3β,5β,12β)-3-[(*O*-2,6-dideoxy-β-*D*-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-*D*-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-*D*-*ribo*-hexopyranosyl)oxy]-12,14-dihydroxy-card-20(22)-enolide. Its molecular formula is $C_{41}H_{64}O_{14}$, its molecular weight is 780.95, and its structural formula is:



Digoxin exists as odorless white crystals that melt with decomposition above 230°C. The drug is practically insoluble in water and in ether; slightly soluble in diluted (50%) alcohol and in chloroform; and freely soluble in pyridine.

LANOXIN is supplied as 125-mcg (0.125-mg) or 250-mcg (0.25-mg) tablets for oral administration. Each tablet contains the labeled amount of digoxin USP and the following inactive ingredients: corn and potato starches, lactose, and magnesium stearate. In addition, the dyes used in the 125-mcg (0.125-mg) tablets are D&C Yellow No. 10 and FD&C Yellow No. 6.

CLINICAL PHARMACOLOGY: Mechanism of Action: Digoxin inhibits sodium-potassium ATPase, an enzyme that regulates the quantity of sodium and potassium inside cells. Inhibition of the enzyme leads to an increase in the intracellular concentration of sodium and thus (by stimulation of sodium-calcium exchange) an increase in the intracellular concentration of calcium. The beneficial effects of digoxin result from direct actions on cardiac muscle, as well as

indirect actions on the cardiovascular system mediated by effects on the autonomic nervous system. The autonomic effects include: (1) a vagomimetic action, which is responsible for the effects of digoxin on the sinoatrial and atrioventricular (AV) nodes; and (2) baroreceptor sensitization, which results in increased afferent inhibitory activity and reduced activity of the sympathetic nervous system and renin-angiotensin system for any given increment in mean arterial pressure. The pharmacologic consequences of these direct and indirect effects are: (1) an increase in the force and velocity of myocardial systolic contraction (positive inotropic action); (2) a decrease in the degree of activation of the sympathetic nervous system and renin-angiotensin system (neurohormonal deactivating effect); and (3) slowing of the heart rate and decreased conduction velocity through the AV node (vagomimetic effect). The effects of digoxin in heart failure are mediated by its positive inotropic and neurohormonal deactivating effects, whereas the effects of the drug in atrial arrhythmias are related to its vagomimetic actions. In high doses, digoxin increases sympathetic outflow from the central nervous system (CNS). This increase in sympathetic activity may be an important factor in digitalis toxicity.

Pharmacokinetics: ***Absorption:*** Following oral administration, peak serum concentrations of digoxin occur at 1 to 3 hours. Absorption of digoxin from LANOXIN Tablets has been demonstrated to be 60% to 80% complete compared to an identical intravenous dose of digoxin (absolute bioavailability) or LANOXICAPS® (relative bioavailability). When LANOXIN Tablets are taken after meals, the rate of absorption is slowed, but the total amount of digoxin absorbed is usually unchanged. When taken with meals high in bran fiber, however, the amount absorbed from an oral dose may be reduced. Comparisons of the systemic availability and equivalent doses for oral preparations of LANOXIN are shown in Table 1.

Table 1: Comparisons of the Systemic Availability and Equivalent Doses for Oral Preparations of LANOXIN

Product	Absolute Bioavailability	Equivalent Doses (mcg)* Among Dosage Forms			
LANOXIN Tablets	60 - 80%	62.5	125	250	500
LANOXIN Elixir Pediatric	70 - 85%	62.5	125	250	500
LANOXICAPS®	90 - 100%	50	100	200	400
LANOXIN Injection/IV	100%	50	100	200	400

* For example, 125-mcg LANOXIN Tablets equivalent to 125-mcg LANOXIN Elixir Pediatric equivalent to 100-mcg LANOXICAPS equivalent to 100-mcg LANOXIN Injection/IV.

In some patients, orally administered digoxin is converted to inactive reduction products (e.g., dihydrodigoxin) by colonic bacteria in the gut. Data suggest that one in ten patients treated with digoxin tablets will degrade 40% or more of the ingested dose. As a result, certain antibiotics may increase the absorption of digoxin in such patients. Although inactivation of these bacteria by antibiotics is rapid, the serum digoxin concentration will rise at a rate consistent with the elimination half-life of digoxin. The magnitude of rise in serum digoxin concentration relates to the extent of bacterial inactivation, and may be as much as two-fold in some cases.

Distribution: Following drug administration, a 6- to 8-hour tissue distribution phase is observed. This is followed by a much more gradual decline in the serum concentration of the drug, which is dependent on the elimination of digoxin from the body. The peak height and slope of the early portion (absorption/distribution phases) of the serum concentration-time curve are

dependent upon the route of administration and the absorption characteristics of the formulation. Clinical evidence indicates that the early high serum concentrations do not reflect the concentration of digoxin at its site of action, but that with chronic use, the steady-state post-distribution serum concentrations are in equilibrium with tissue concentrations and correlate with pharmacologic effects. In individual patients, these post-distribution serum concentrations may be useful in evaluating therapeutic and toxic effects (see DOSAGE AND ADMINISTRATION: Serum Digoxin Concentrations).

Digoxin is concentrated in tissues and therefore has a large apparent volume of distribution. Digoxin crosses both the blood-brain barrier and the placenta. At delivery, the serum digoxin concentration in the newborn is similar to the serum concentration in the mother. Approximately 25% of digoxin in the plasma is bound to protein. Serum digoxin concentrations are not significantly altered by large changes in fat tissue weight, so that its distribution space correlates best with lean (i.e., ideal) body weight, not total body weight.

Metabolism: Only a small percentage (16%) of a dose of digoxin is metabolized. The end metabolites, which include 3 ß-digoxigenin, 3-keto-digoxigenin, and their glucuronide and sulfate conjugates, are polar in nature and are postulated to be formed via hydrolysis, oxidation, and conjugation. The metabolism of digoxin is not dependent upon the cytochrome P-450 system, and digoxin is not known to induce or inhibit the cytochrome P-450 system.

Excretion: Elimination of digoxin follows first-order kinetics (that is, the quantity of digoxin eliminated at any time is proportional to the total body content). Following intravenous administration to healthy volunteers, 50% to 70% of a digoxin dose is excreted unchanged in the urine. Renal excretion of digoxin is proportional to glomerular filtration rate and is largely independent of urine flow. In healthy volunteers with normal renal function, digoxin has a half-life of 1.5 to 2.0 days. The half-life in anuric patients is prolonged to 3.5 to 5 days. Digoxin is not effectively removed from the body by dialysis, exchange transfusion, or during cardiopulmonary bypass because most of the drug is bound to tissue and does not circulate in the blood.

Special Populations: Race differences in digoxin pharmacokinetics have not been formally studied. Because digoxin is primarily eliminated as unchanged drug via the kidney and because there are no important differences in creatinine clearance among races, pharmacokinetic differences due to race are not expected.

The clearance of digoxin can be primarily correlated with renal function as indicated by creatinine clearance. The Cockcroft and Gault formula for estimation of creatinine clearance includes age, body weight, and gender. Table 5 that provides the usual daily maintenance dose requirements of LANOXIN Tablets based on creatinine clearance (per 70 kg) is presented in the DOSAGE AND ADMINISTRATION section.

Plasma digoxin concentration profiles in patients with acute hepatitis generally fell within the range of profiles in a group of healthy subjects.

Pharmacodynamic and Clinical Effects: The times to onset of pharmacologic effect and to peak effect of preparations of LANOXIN are shown in Table 2.

Table 2: Times to Onset of Pharmacologic Effect and to Peak Effect of Preparations of LANOXIN

Product	Time to Onset of Effect*	Time to Peak Effect*
LANOXIN Tablets	0.5 - 2 hours	2 - 6 hours
LANOXIN Elixir Pediatric	0.5 - 2 hours	2 - 6 hours
LANOXICAPS	0.5 - 2 hours	2 - 6 hours
LANOXIN Injection/IV	5 - 30 minutes†	1 - 4 hours

* Documented for ventricular response rate in atrial fibrillation, inotropic effects and electrocardiographic changes.

† Depending upon rate of infusion.

Hemodynamic Effects: Digoxin produces hemodynamic improvement in patients with heart failure. Short- and long-term therapy with the drug increases cardiac output and lowers pulmonary artery pressure, pulmonary capillary wedge pressure, and systemic vascular resistance. These hemodynamic effects are accompanied by an increase in the left ventricular ejection fraction and a decrease in end-systolic and end-diastolic dimensions.

Chronic Heart Failure: Two 12-week, double-blind, placebo-controlled studies enrolled 178 (RADIANCE trial) and 88 (PROVED trial) patients with NYHA class II or III heart failure previously treated with digoxin, a diuretic, and an ACE inhibitor (RADIANCE only) and randomized them to placebo or treatment with LANOXIN. Both trials demonstrated better preservation of exercise capacity in patients randomized to LANOXIN. Continued treatment with LANOXIN reduced the risk of developing worsening heart failure, as evidenced by heart failure-related hospitalizations and emergency care and the need for concomitant heart failure therapy. The larger study also showed treatment-related benefits in NYHA class and patients' global assessment. In the smaller trial, these trended in favor of a treatment benefit.

The Digitalis Investigation Group (DIG) main trial was a multicenter, randomized, double-blind, placebo-controlled mortality study of 6801 patients with heart failure and left ventricular ejection fraction ≤0.45. At randomization, 67% were NYHA class I or II, 71% had heart failure of ischemic etiology, 44% had been receiving digoxin, and most were receiving concomitant ACE inhibitor (94%) and diuretic (82%). Patients were randomized to placebo or LANOXIN, the dose of which was adjusted for the patient's age, sex, lean body weight, and serum creatinine (see DOSAGE AND ADMINISTRATION), and followed for up to 58 months (median 37 months). The median daily dose prescribed was 0.25 mg. Overall all-cause mortality was 35% with no difference between groups (95% confidence limits for relative risk of 0.91 to 1.07). LANOXIN was associated with a 25% reduction in the number of hospitalizations for heart failure, a 28% reduction in the risk of a patient having at least one hospitalization for heart failure, and a 6.5% reduction in total hospitalizations (for any cause).

Use of LANOXIN was associated with a trend to increase time to all-cause death or hospitalization. The trend was evident in subgroups of patients with mild heart failure as well as more severe disease, as shown in Table 3. Although the effect on all-cause death or hospitalization was not statistically significant, much of the apparent benefit derived from effects on mortality and hospitalization attributed to heart failure.

Table 3: Subgroup Analyses of Mortality and Hospitalization During the First Two Years Following Randomization

		Risk of All-Cause Mortality or All-Cause Hospitalization*			Risk of HF-Related Mortality or HF-Related Hospitalization*		
	n	Placebo	LANOXIN	Relative risk†	Placebo	LANOXIN	Relative risk†
All patients (EF ≤0.45)	6801	604	593	0.94 (0.88-1.00)	294	217	0.69 (0.63-0.76)
NYHA I/II	4571	549	541	0.96 (0.89-1.04)	242	178	0.70 (0.62-0.80)
EF 0.25-0.45	4543	568	571	0.99 (0.91-1.07)	244	190	0.74 (0.66-0.84)
CTR ≤0.55	4455	561	563	0.98 (0.91-1.06)	239	180	0.71 (0.63-0.81)
NYHA III / IV	2224	719	696	0.88 (0.80-0.97)	402	295	0.65 (0.57-0.75)
EF <0.25	2258	677	637	0.84 (0.76-0.93)	394	270	0.61 (0.53-0.71)
CTR >0.55	2346	687	650	0.85 (0.77-0.94)	398	287	0.65 (0.57-0.75)
EF >0.45‡	987	571	585	1.04 (0.88-1.23)	179	136	0.72 (0.53-0.99)

* Number of patients with an event during the first 2 years per 1000 randomized patients.
† Relative risk (95% confidence interval).
‡ DIG Ancillary Study.

In situations where there is no statistically significant benefit of treatment evident from a trial's primary endpoint, results pertaining to a secondary endpoint should be interpreted cautiously.

Chronic Atrial Fibrillation: In patients with chronic atrial fibrillation, digoxin slows rapid ventricular response rate in a linear dose-response fashion from 0.25 to 0.75 mg/day. Digoxin should not be used for the treatment of multifocal atrial tachycardia.

INDICATIONS AND USAGE:

Heart Failure: LANOXIN is indicated for the treatment of mild to moderate heart failure. LANOXIN increases left ventricular ejection fraction and improves heart failure symptoms as evidenced by exercise capacity and heart failure-related hospitalizations and emergency care, while having no effect on mortality. Where possible, LANOXIN should be used with a diuretic and an angiotensin-converting enzyme inhibitor, but an optimal order for starting these three drugs cannot be specified.

Atrial Fibrillation: LANOXIN is indicated for the control of ventricular response rate in patients with chronic atrial fibrillation.

CONTRAINDICATIONS: Digitalis glycosides are contraindicated in patients with ventricular fibrillation or in patients with a known hypersensitivity to digoxin. A hypersensitivity reaction to other digitalis preparations usually constitutes a contraindication to digoxin.

WARNINGS:

Sinus Node Disease and AV Block: Because digoxin slows sinoatrial and AV conduction, the drug commonly prolongs the PR interval. The drug may cause severe sinus bradycardia or sinoatrial block in patients with pre-existing sinus node disease and may cause advanced or complete heart block in patients with pre-existing incomplete AV block. In such patients consideration should be given to the insertion of a pacemaker before treatment with digoxin.

Accessory AV Pathway (Wolff-Parkinson-White Syndrome): After intravenous digoxin therapy, some patients with paroxysmal atrial fibrillation or flutter and a coexisting accessory AV pathway have developed increased antegrade conduction across the accessory pathway bypassing the AV node, leading to a very rapid ventricular response or ventricular fibrillation. Unless conduction down the accessory pathway has been blocked (either pharmacologically or by surgery), digoxin should not be used in such patients. The treatment of paroxysmal supraventricular tachycardia in such patients is usually direct-current cardioversion.

Use in Patients with Preserved Left Ventricular Systolic Function: Patients with certain disorders involving heart failure associated with preserved left ventricular ejection fraction may be particularly susceptible to toxicity of the drug. Such disorders include restrictive cardiomyopathy, constrictive pericarditis, amyloid heart disease, and acute cor pulmonale. Patients with idiopathic hypertrophic subaortic stenosis may have worsening of the outflow obstruction due to the inotropic effects of digoxin.

PRECAUTIONS:

Use in Patients with Impaired Renal Function: Digoxin is primarily excreted by the kidneys; therefore, patients with impaired renal function require smaller than usual maintenance doses of digoxin (see DOSAGE AND ADMINISTRATION). Because of the prolonged elimination half-life, a longer period of time is required to achieve an initial or new steady-state serum concentration in patients with renal impairment than in patients with normal renal function. If appropriate care is not taken to reduce the dose of digoxin, such patients are at high risk for toxicity, and toxic effects will last longer in such patients than in patients with normal renal function.

Use in Patients with Electrolyte Disorders: In patients with hypokalemia or hypomagnesemia, toxicity may occur despite serum digoxin concentrations below 2.0 ng/mL, because potassium or magnesium depletion sensitizes the myocardium to digoxin. Therefore, it is desirable to maintain normal serum potassium and magnesium concentrations in patients being treated with digoxin. Deficiencies of these electrolytes may result from malnutrition, diarrhea, or prolonged vomiting, as well as the use of the following drugs or procedures: diuretics, amphotericin B, corticosteroids, antacids, dialysis, and mechanical suction of gastrointestinal secretions.

Hypercalcemia from any cause predisposes the patient to digitalis toxicity. Calcium, particularly when administered rapidly by the intravenous route, may produce serious arrhythmias in digitalized patients. On the other hand, hypocalcemia can nullify the effects of digoxin in humans; thus, digoxin may be ineffective until serum calcium is restored to normal.

These interactions are related to the fact that digoxin affects contractility and excitability of the heart in a manner similar to that of calcium.

Use in Thyroid Disorders and Hypermetabolic States: Hypothyroidism may reduce the requirements for digoxin. Heart failure and/or atrial arrhythmias resulting from hypermetabolic or hyperdynamic states (e.g., hyperthyroidism, hypoxia, or arteriovenous shunt) are best treated by addressing the underlying condition. Atrial arrhythmias associated with hypermetabolic states are particularly resistant to digoxin treatment. Care must be taken to avoid toxicity if digoxin is used.

Use in Patients with Acute Myocardial Infarction: Digoxin should be used with caution in patients with acute myocardial infarction. The use of inotropic drugs in some patients in this setting may result in undesirable increases in myocardial oxygen demand and ischemia.

Use During Electrical Cardioversion: It may be desirable to reduce the dose of digoxin for 1 to 2 days prior to electrical cardioversion of atrial fibrillation to avoid the induction of ventricular arrhythmias, but physicians must consider the consequences of increasing the ventricular response if digoxin is withdrawn. If digitalis toxicity is suspected, elective cardioversion should be delayed. If it is not prudent to delay cardioversion, the lowest possible energy level should be selected to avoid provoking ventricular arrhythmias.

Laboratory Test Monitoring: Patients receiving digoxin should have their serum electrolytes and renal function (serum creatinine concentrations) assessed periodically; the frequency of assessments will depend on the clinical setting. For discussion of serum digoxin concentrations, see DOSAGE AND ADMINISTRATION section.

Drug Interactions: Potassium-depleting *diuretics* are a major contributing factor to digitalis toxicity. *Calcium*, particularly if administered rapidly by the intravenous route, may produce serious arrhythmias in digitalized patients. *Quinidine, verapamil, amiodarone, propafenone, indomethacin*, *itraconazole*, *alprazolam,* and *spironolactone* raise the serum digoxin concentration due to a reduction in clearance and/or in volume of distribution of the drug, with the implication that digitalis intoxication may result. *Erythromycin* and *clarithromycin* (and possibly other *macrolide antibiotics*) and *tetracycline* may increase digoxin absorption in patients who inactivate digoxin by bacterial metabolism in the lower intestine, so that digitalis intoxication may result (see CLINICAL PHARMACOLOGY: Absorption). *Propantheline* and *diphenoxylate,* by decreasing gut motility, may increase digoxin absorption. *Antacids, kaolin-pectin, sulfasalazine, neomycin, cholestyramine,* certain *anticancer drugs*, and *metoclopramide* may interfere with intestinal digoxin absorption, resulting in unexpectedly low serum concentrations. *Rifampin* may decrease serum digoxin concentration, especially in patients with renal dysfunction, by increasing the non-renal clearance of digoxin. There have been inconsistent reports regarding the effects of other drugs [e.g., *quinine, penicillamine*] on serum digoxin concentration. *Thyroid* administration to a digitalized, hypothyroid patient may increase the dose requirement of digoxin. Concomitant use of digoxin and *sympathomimetics* increases the risk of cardiac arrhythmias. *Succinylcholine* may cause a sudden extrusion of potassium from muscle cells, and may thereby cause arrhythmias in digitalized patients. Although beta-adrenergic blockers or calcium channel blockers and digoxin may be useful in combination to control atrial fibrillation, their additive effects on AV node conduction can result in advanced or complete heart block.

Due to the considerable variability of these interactions, the dosage of digoxin should be individualized when patients receive these medications concurrently. Furthermore, caution

should be exercised when combining digoxin with any drug that may cause a significant deterioration in renal function, since a decline in glomerular filtration or tubular secretion may impair the excretion of digoxin.

Drug/Laboratory Test Interactions: The use of therapeutic doses of digoxin may cause prolongation of the PR interval and depression of the ST segment on the electrocardiogram. Digoxin may produce false positive ST-T changes on the electrocardiogram during exercise testing. These electrophysiologic effects reflect an expected effect of the drug and are not indicative of toxicity.

Carcinogenesis, Mutagenesis, Impairment of Fertility: There have been no long-term studies performed in animals to evaluate carcinogenic potential, nor have studies been conducted to assess the mutagenic potential of digoxin or its potential to affect fertility.

Pregnancy: ***Teratogenic Effects:*** Pregnancy Category C. Animal reproduction studies have not been conducted with digoxin. It is also not known whether digoxin can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. Digoxin should be given to a pregnant woman only if clearly needed.

Nursing Mothers: Studies have shown that digoxin concentrations in the mother's serum and milk are similar. However, the estimated exposure of a nursing infant to digoxin via breast feeding will be far below the usual infant maintenance dose. Therefore, this amount should have no pharmacologic effect upon the infant. Nevertheless, caution should be exercised when digoxin is administered to a nursing woman.

Pediatric Use: Newborn infants display considerable variability in their tolerance to digoxin. Premature and immature infants are particularly sensitive to the effects of digoxin, and the dosage of the drug must not only be reduced but must be individualized according to their degree of maturity. Digitalis glycosides can cause poisoning in children due to accidental ingestion.

Geriatric Use: The majority of clinical experience gained with digoxin has been in the elderly population. This experience has not identified differences in response or adverse effects between the elderly and younger patients. However, this drug is known to be substantially excreted by the kidney, and the risk of toxic reactions to this drug may be greater in patients with impaired renal function. Because elderly patients are more likely to have decreased renal function, care should be taken in dose selection, which should be based on renal function, and it may be useful to monitor renal function (see DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS: In general, the adverse reactions of digoxin are dose-dependent and occur at doses higher than those needed to achieve a therapeutic effect. Hence, adverse reactions are less common when digoxin is used within the recommended dose range or therapeutic serum concentration range and when there is careful attention to concurrent medications and conditions.

Because some patients may be particularly susceptible to side effects with digoxin, the dosage of the drug should always be selected carefully and adjusted as the clinical condition of the patient warrants. In the past, when high doses of digoxin were used and little attention was paid to clinical status or concurrent medications, adverse reactions to digoxin were more frequent and severe. Cardiac adverse reactions accounted for about one-half, gastrointestinal disturbances for about one-fourth, and CNS and other toxicity for about one-fourth of these adverse reactions. However, available evidence suggests that the incidence and severity of digoxin toxicity has decreased substantially in recent years. In recent controlled clinical trials, in patients with predominantly mild to moderate heart failure, the incidence of adverse experiences was

comparable in patients taking digoxin and in those taking placebo. In a large mortality trial, the incidence of hospitalization for suspected digoxin toxicity was 2% in patients taking LANOXIN compared to 0.9% in patients taking placebo. In this trial, the most common manifestations of digoxin toxicity included gastrointestinal and cardiac disturbances; CNS manifestations were less common.

Adults: ***Cardiac:*** Therapeutic doses of digoxin may cause heart block in patients with pre-existing sinoatrial or AV conduction disorders; heart block can be avoided by adjusting the dose of digoxin. Prophylactic use of a cardiac pacemaker may be considered if the risk of heart block is considered unacceptable. High doses of digoxin may produce a variety of rhythm disturbances, such as first-degree, second-degree (Wenckebach), or third-degree heart block (including asystole); atrial tachycardia with block; AV dissociation; accelerated junctional (nodal) rhythm; unifocal or multiform ventricular premature contractions (especially bigeminy or trigeminy); ventricular tachycardia; and ventricular fibrillation. Digoxin produces PR prolongation and ST segment depression which should not by themselves be considered digoxin toxicity. Cardiac toxicity can also occur at therapeutic doses in patients who have conditions which may alter their sensitivity to digoxin (see WARNINGS and PRECAUTIONS).

Gastrointestinal: Digoxin may cause anorexia, nausea, vomiting, and diarrhea. Rarely, the use of digoxin has been associated with abdominal pain, intestinal ischemia, and hemorrhagic necrosis of the intestines.

CNS: Digoxin can produce visual disturbances (blurred or yellow vision), headache, weakness, dizziness, apathy, confusion, and mental disturbances (such as anxiety, depression, delirium, and hallucination).

Other: Gynecomastia has been occasionally observed following the prolonged use of digoxin. Thrombocytopenia and maculopapular rash and other skin reactions have been rarely observed.

Table 4 summarizes the incidence of those adverse experiences listed above for patients treated with LANOXIN Tablets or placebo from two randomized, double-blind, placebo-controlled withdrawal trials. Patients in these trials were also receiving diuretics with or without angiotensin-converting enzyme inhibitors. These patients had been stable on digoxin, and were randomized to digoxin or placebo. The results shown in Table 4 reflect the experience in patients following dosage titration with the use of serum digoxin concentrations and careful follow-up. These adverse experiences are consistent with results from a large, placebo-controlled mortality trial (DIG trial) wherein over half the patients were not receiving digoxin prior to enrollment.

Table 4: Adverse Experiences In Two Parallel, Double-Blind, Placebo-Controlled Withdrawal Trials (Number of Patients Reporting)

Adverse Experience	Digoxin Patients (n = 123)	Placebo Patients (n = 125)
Cardiac		
Palpitation	1	4
Ventricular extrasystole	1	1
Tachycardia	2	1
Heart arrest	1	1
Gastrointestinal		
Anorexia	1	4
Nausea	4	2
Vomiting	2	1
Diarrhea	4	1
Abdominal pain	0	6
CNS		
Headache	4	4
Dizziness	6	5
Mental disturbances	5	1
Other		
Rash	2	1
Death	4	3

Infants and Children: The side effects of digoxin in infants and children differ from those seen in adults in several respects. Although digoxin may produce anorexia, nausea, vomiting, diarrhea, and CNS disturbances in young patients, these are rarely the initial symptoms of overdosage. Rather, the earliest and most frequent manifestation of excessive dosing with digoxin in infants and children is the appearance of cardiac arrhythmias, including sinus bradycardia. In children, the use of digoxin may produce any arrhythmia. The most common are conduction disturbances or supraventricular tachyarrhythmias, such as atrial tachycardia (with or without block) and junctional (nodal) tachycardia. Ventricular arrhythmias are less common. Sinus bradycardia may be a sign of impending digoxin intoxication, especially in infants, even in the absence of first-degree heart block. Any arrhythmia or alteration in cardiac conduction that develops in a child taking digoxin should be assumed to be caused by digoxin, until further evaluation proves otherwise.

OVERDOSAGE:

Treatment of Adverse Reactions Produced by Overdosage: Digoxin should be temporarily discontinued until the adverse reaction resolves. Every effort should also be made to correct factors that may contribute to the adverse reaction (such as electrolyte disturbances or concurrent medications). Once the adverse reaction has resolved, therapy with digoxin may be reinstituted, following a careful reassessment of dose.

Withdrawal of digoxin may be all that is required to treat the adverse reaction. However, when the primary manifestation of digoxin overdosage is a cardiac arrhythmia, additional therapy may be needed.

If the rhythm disturbance is a symptomatic bradyarrhythmia or heart block, consideration should be given to the reversal of toxicity with DIGIBIND® [Digoxin Immune Fab (Ovine)] (see Massive Digitalis Overdosage subsection), the use of atropine, or the insertion of a temporary cardiac pacemaker. However, asymptomatic bradycardia or heart block related to digoxin may require only temporary withdrawal of the drug and cardiac monitoring of the patient.

If the rhythm disturbance is a ventricular arrhythmia, consideration should be given to the correction of electrolyte disorders, particularly if hypokalemia (seeAdministration of Potassium subsection) or hypomagnesemia is present. DIGIBIND is a specific antidote for digoxin and may be used to reverse potentially life-threatening ventricular arrhythmias due to digoxin overdosage.

Administration of Potassium: Every effort should be made to maintain the serum potassium concentration between 4.0 and 5.5 mmol/L. Potassium is usually administered orally, but when correction of the arrhythmia is urgent and the serum potassium concentration is low, potassium may be administered cautiously by the intravenous route. The electrocardiogram should be monitored for any evidence of potassium toxicity (e.g., peaking of T waves) and to observe the effect on the arrhythmia. Potassium salts may be dangerous in patients who manifest bradycardia or heart block due to digoxin (unless primarily related to supraventricular tachycardia) and in the setting of massive digitalis overdosage (see Massive Digitalis Overdosage subsection).

Massive Digitalis Overdosage: Manifestations of life-threatening toxicity include ventricular tachycardia or ventricular fibrillation, or progressive bradyarrhythmias, or heart block. The administration of more than 10 mg of digoxin in a previously healthy adult, or more than 4 mg in a previously healthy child, or a steady-state serum concentration greater than 10 ng/mL often results in cardiac arrest.

DIGIBIND should be used to reverse the toxic effects of ingestion of a massive overdose. The decision to administer DIGIBIND to a patient who has ingested a massive dose of digoxin but who has not yet manifested life-threatening toxicity should depend on the likelihood that life-threatening toxicity will occur (see above).

Patients with massive digitalis ingestion should receive large doses of activated charcoal to prevent absorption and bind digoxin in the gut during enteroenteric recirculation. Emesis or gastric lavage may be indicated especially if ingestion has occurred within 30 minutes of the patient's presentation at the hospital. Emesis should not be induced in patients who are obtunded. If a patient presents more than 2 hours after ingestion or already has toxic manifestations, it may be unsafe to induce vomiting or attempt passage of a gastric tube, because such maneuvers may induce an acute vagal episode that can worsen digitalis-related arrhythmias.

Severe digitalis intoxication can cause a massive shift of potassium from inside to outside the cell, leading to life-threatening hyperkalemia. The administration of potassium supplements in the setting of massive intoxication may be hazardous and should be avoided. Hyperkalemia caused by massive digitalis toxicity is best treated with DIGIBIND; initial treatment with glucose and insulin may also be required if hyperkalemia itself is acutely life-threatening.

DOSAGE AND ADMINISTRATION:

General: Recommended dosages of digoxin may require considerable modification because of individual sensitivity of the patient to the drug, the presence of associated conditions, or the use

of concurrent medications. In selecting a dose of digoxin, the following factors must be considered:

1. The body weight of the patient. Doses should be calculated based upon lean (i.e., ideal) body weight.
2. The patient's renal function, preferably evaluated on the basis of estimated creatinine clearance.
3. The patient's age. Infants and children require different doses of digoxin than adults. Also, advanced age may be indicative of diminished renal function even in patients with normal serum creatinine concentration (i.e., below 1.5 mg/dL).
4. Concomitant disease states, concurrent medications, or other factors likely to alter the pharmacokinetic or pharmacodynamic profile of digoxin (see PRECAUTIONS).

Serum Digoxin Concentrations: In general, the dose of digoxin used should be determined on clinical grounds. However, measurement of serum digoxin concentrations can be helpful to the clinician in determining the adequacy of digoxin therapy and in assigning certain probabilities to the likelihood of digoxin intoxication. About two-thirds of adults considered adequately digitalized (without evidence of toxicity) have serum digoxin concentrations ranging from 0.8 to 2.0 ng/mL. However, digoxin may produce clinical benefits even at serum concentrations below this range. About two-thirds of adult patients with clinical toxicity have serum digoxin concentrations greater than 2.0 ng/mL. However, since one-third of patients with clinical toxicity have concentrations less than 2.0 ng/mL, values below 2.0 ng/mL do not rule out the possibility that a certain sign or symptom is related to digoxin therapy. Rarely, there are patients who are unable to tolerate digoxin at serum concentrations below 0.8 ng/mL. Consequently, the serum concentration of digoxin should always be interpreted in the overall clinical context, and an isolated measurement should not be used alone as the basis for increasing or decreasing the dose of the drug.

To allow adequate time for equilibration of digoxin between serum and tissue, sampling of serum concentrations should be done just before the next scheduled dose of the drug. If this is not possible, sampling should be done at least 6 to 8 hours after the last dose, regardless of the route of administration or the formulation used. On a once-daily dosing schedule, the concentration of digoxin will be 10% to 25% lower when sampled at 24 versus 8 hours, depending upon the patient's renal function. On a twice-daily dosing schedule, there will be only minor differences in serum digoxin concentrations whether sampling is done at 8 or 12 hours after a dose.

If a discrepancy exists between the reported serum concentration and the observed clinical response, the clinician should consider the following possibilities:

1. Analytical problems in the assay procedure.
2. Inappropriate serum sampling time.
3. Administration of a digitalis glycoside other than digoxin.
4. Conditions (described in WARNINGS and PRECAUTIONS) causing an alteration in the sensitivity of the patient to digoxin.
5. Serum digoxin concentration may decrease acutely during periods of exercise without any associated change in clinical efficacy due to increased binding of digoxin to skeletal muscle.

Heart Failure: ***Adults:*** Digitalization may be accomplished by either of two general approaches that vary in dosage and frequency of administration, but reach the same endpoint in terms of total amount of digoxin accumulated in the body.

1. If rapid digitalization is considered medically appropriate, it may be achieved by administering a loading dose based upon projected peak digoxin body stores. Maintenance dose can be calculated as a percentage of the loading dose.
2. More gradual digitalization may be obtained by beginning an appropriate maintenance dose, thus allowing digoxin body stores to accumulate slowly. Steady-state serum digoxin concentrations will be achieved in approximately five half-lives of the drug for the individual patient. Depending upon the patient's renal function, this will take between 1 and 3 weeks.

Rapid Digitalization with a Loading Dose: Peak digoxin body stores of 8 to 12 mcg/kg should provide therapeutic effect with minimum risk of toxicity in most patients with heart failure and normal sinus rhythm. Because of altered digoxin distribution and elimination, projected peak body stores for patients with renal insufficiency should be conservative (i.e., 6 to 10 mcg/kg) [see PRECAUTIONS].

The loading dose should be administered in several portions, with roughly half the total given as the first dose. Additional fractions of this planned total dose may be given at 6- to 8-hour intervals, **with careful assessment of clinical response before each additional dose.**

If the patient's clinical response necessitates a change from the calculated loading dose of digoxin, then calculation of the maintenance dose should be based upon the amount actually given.

A single initial dose of 500 to 750 mcg (0.5 to 0.75 mg) of LANOXIN Tablets usually produces a detectable effect in 0.5 to 2 hours that becomes maximal in 2 to 6 hours. Additional doses of 125 to 375 mcg (0.125 to 0.375 mg) may be given cautiously at 6- to 8-hour intervals until clinical evidence of an adequate effect is noted. The usual amount of LANOXIN Tablets that a 70-kg patient requires to achieve 8 to 12 mcg/kg peak body stores is 750 to 1250 mcg (0.75 to 1.25 mg).

LANOXIN Injection is frequently used to achieve rapid digitalization, with conversion to LANOXIN Tablets or LANOXICAPS for maintenance therapy. If patients are switched from intravenous to oral digoxin formulations, allowances must be made for differences in bioavailability when calculating maintenance dosages (see Table 1, CLINICAL PHARMACOLOGY).

Maintenance Dosing: The doses of digoxin used in controlled trials in patients with heart failure have ranged from 125 to 500 mcg (0.125 to 0.5 mg) once daily. In these studies, the digoxin dose has been generally titrated according to the patient's age, lean body weight, and renal function. Therapy is generally initiated at a dose of 250 mcg (0.25 mg) once daily in patients under age 70 with good renal function, at a dose of 125 mcg (0.125 mg) once daily in patients over age 70 or with impaired renal function, and at a dose of 62.5 mcg (0.0625 mg) in patients with marked renal impairment. Doses may be increased every 2 weeks according to clinical response.

In a subset of approximately 1800 patients enrolled in the DIG trial (wherein dosing was based on an algorithm similar to that in Table 5) the mean (± SD) serum digoxin concentrations at 1 month and 12 months were 1.01 ± 0.47 ng/mL and 0.97 ± 0.43 ng/mL, respectively.

The maintenance dose should be based upon the percentage of the peak body stores lost each day through elimination. The following formula has had wide clinical use:

Maintenance Dose = Peak Body Stores (i.e., Loading Dose) x % Daily Loss/100

Where: % Daily Loss = 14 + Ccr/5
(Ccr is creatinine clearance, corrected to 70 kg body weight or 1.73 m^2 body surface area.)

Table 5 provides average daily maintenance dose requirements of LANOXIN Tablets for patients with heart failure based upon lean body weight and renal function:

Table 5: Usual Daily Maintenance Dose Requirements (mcg) of LANOXIN for Estimated Peak Body Stores of 10 mcg/kg

Corrected Ccr (mL/min per 70 kg)*	Lean Body Weight kg	50	60	70	80	90	100	Number of Days Before Steady State Achieved†
	lb	110	132	154	176	198	220	
0		62.5‡	125	125	125	187.5	187.5	22
10		125	125	125	187.5	187.5	187.5	19
20		125	125	187.5	187.5	187.5	250	16
30		125	187.5	187.5	187.5	250	250	14
40		125	187.5	187.5	250	250	250	13
50		187.5	187.5	250	250	250	250	12
60		187.5	187.5	250	250	250	375	11
70		187.5	250	250	250	250	375	10
80		187.5	250	250	250	375	375	9
90		187.5	250	250	250	375	500	8
100		250	250	250	375	375	500	7

*Ccr is creatinine clearance, corrected to 70 kg body weight or 1.73 m^2 body surface area. *For adults,* if only serum creatinine concentrations (Scr) are available, a Ccr (corrected to 70 kg body weight) may be estimated in men as (140 - Age)/Scr. For women, this result should be multiplied by 0.85. *Note:* This equation cannot be used for estimating creatinine clearance in infants or children.

†If no loading dose administered.

‡62.5 mcg = 0.0625 mg

Example: Based on Table 5, a patient in heart failure with an estimated lean body weight of 70 kg and a Ccr of 60 mL/min should be given a dose of 250 mcg (0.25 mg) daily of LANOXIN Tablets, usually taken after the morning meal. If no loading dose is administered, steady-state serum concentrations in this patient should be anticipated at approximately 11 days.

Infants and Children: In general, divided daily dosing is recommended for infants and young children (under age 10). In the newborn period, renal clearance of digoxin is diminished and suitable dosage adjustments must be observed. This is especially pronounced in the premature infant. Beyond the immediate newborn period, children generally require proportionally larger doses than adults on the basis of body weight or body surface area. Children over 10 years of age require adult dosages in proportion to their body weight. Some researchers have suggested that infants and young children tolerate slightly higher serum concentrations than do adults.

Daily maintenance doses for each age group are given in Table 6 and should provide therapeutic effects with minimum risk of toxicity in most patients with heart failure and normal sinus rhythm. These recommendations assume the presence of normal renal function:

Table 6: Daily Maintenance Doses in Children with Normal Renal Function

Age	Daily Maintenance Dose (mcg/kg)
2 to 5 Years	10 to 15
5 to 10 Years	7 to 10
Over 10 Years	3 to 5

In children with renal disease, digoxin must be carefully titrated based upon clinical response.

It cannot be overemphasized that both the adult and pediatric dosage guidelines provided are based upon average patient response and substantial individual variation can be expected. Accordingly, ultimate dosage selection must be based upon clinical assessment of the patient.

Atrial Fibrillation: Peak digoxin body stores larger than the 8 to 12 mcg/kg required for most patients with heart failure and normal sinus rhythm have been used for control of ventricular rate in patients with atrial fibrillation. Doses of digoxin used for the treatment of chronic atrial fibrillation should be titrated to the minimum dose that achieves the desired ventricular rate control without causing undesirable side effects. Data are not available to establish the appropriate resting or exercise target rates that should be achieved.

Dosage Adjustment When Changing Preparations: The difference in bioavailability between LANOXIN Injection or LANOXICAPS and LANOXIN Elixir Pediatric or LANOXIN Tablets must be considered when changing patients from one dosage form to another.

Doses of 100 mcg (0.1 mg) and 200 mcg (0.2 mg) of LANOXICAPS are approximately equivalent to 125-mcg (0.125-mg) and 250-mcg (0.25-mg) doses of LANOXIN Tablets and Elixir Pediatric, respectively (see Table 1 in CLINICAL PHARMACOLOGY: Pharmacokinetics).

HOW SUPPLIED:

LANOXIN (digoxin) Tablets, Scored 125 mcg (0.125 mg): Bottles of 100 with child-resistant cap (NDC 0173-0242-55) and 1000 (NDC 0173-0242-75); unit dose pack of 100 (NDC 0173-0242-56). Imprinted with LANOXIN and Y3B (yellow).

Store at 25°C (77°F); excursions permitted to 15 to 30°C (59 to 86°F) [see USP Controlled Room Temperature] in a dry place and protect from light.

LANOXIN (digoxin) Tablets, Scored 250 mcg (0.25 mg): Bottles of 100 with child-resistant cap (NDC 0173-0249-55), 1000 (NDC 0173-0249-75), and 5000 (NDC 0173-0249-80); unit dose pack of 100 (NDC 0173-0249-56). Imprinted with LANOXIN and X3A (white).

Store at 25°C (77°F); excursions permitted to 15 to 30°C (59 to 86°F) [see USP Controlled Room Temperature] in a dry place.

GlaxoSmithKline
Research Triangle Park, NC 27709

RL-972

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix D	
Drug substance:	AZD6140
Study Code:	D5130C05265
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Appendix Date:	

Appendix D
Instructions for Collection, Storage and Transport of Blood Samples for Genetic Analysis

1. BLOOD SAMPLE COLLECTION

Ideally, blood should be collected into ***9/10 ml polypropylene tubes*** containing the ***anticoagulant EDTA***. Recommended tubes are detailed in the table below. After collection, blood tubes must be gently ***inverted*** several times to ensure thorough mixing of EDTA with the sample to prevent clotting.

Table of recommended blood tubes for genotyping sample collection

Polypropylene Collection Tube	Part #	Comments
	1066 US 1066.001 UK	SARSTEDT Monovette® EDTA KE - 9ml
	368457 USA/UK	Becton-Dickinson Vacutainer™ K2E - 10ml
	455036 USA/UK	Greiner Bio-one Vacuette® K3E EDTA K3 - 9ml

- ***Glass tubes MUST NOT be used*** as they may break during transport and freeze-thaw cycles.
- ***Heparin MUST NOT be used as an anticoagulant*** as it may interfere with downstream genotyping methodology.

The collection tubes must be labeled with the following information:

- Unique sample ID (compliant with protocol)
- Study ID (and Study Center ID, if available)
- Date of sample collection.

2. STORAGE AT THE STUDY CENTER AND TRANSPORT

After collection, blood samples must be stored appropriately at the site of collection and transported to the Central Handling Facility, or Designated DNA Processing Laboratory, ***as soon as possible***. The table below shows guidelines for sample storage and transport:

Table to show the recommended storage conditions for blood samples immediately after collection

Option	***Storage Temperature at Study Center***	***Maximum Duration***	***Transport Temperature***	***Delivery Time***
1	+ 4°C (fridge)	24 hours	0 - 4°C (ice bricks)	24 hours
2	+ 4°C (fridge)	24 hours	Less than -20°C (dry ice)	24-72 hours
3	-20°C (freezer) or -70°C	Up to 1 month	Less than -20°C (dry ice)	24-72 hours

- **IF BLOOD SAMPLES ARE TO BE STORED AT -20°C OR LESS, NON-FROST FREE FREEZERS MUST BE USED TO PREVENT REPEATED FREEZE-THAW OF BLOOD WHICH MAY REDUCE YIELD & QUALITY OF THE DNA OBTAINED.**

- **SAMPLES MUST NOT BE THAWED AND THEN RE-FROZEN AT ANY POINT**

The Central Handling Facility, or Designated DNA Processing Laboratory, must be notified of the shipment of any samples prior to dispatch. Ideally, the dispatch note must be sent by either fax or email and must contain the following information:

- Study ID, number of samples and list of sample ID's
- Courier name, airway bill number and date of shipment
- Shipment condition (wet ice or dry ice)
- Contact name and address

Considerations should be made to ensure that the samples are delivered during working hours and within 24-72 hours of dispatch.

3. RECOMMENDED PACKAGING INSTRUCTIONS

For safety reasons, all blood samples must be contained. Samples should be individually placed in a clip-lock bag labeled with the sample ID and sealed. Samples may then be batched and again sealed within a second clip-lock bag labeled with the study ID. For ease of further packaging and protection from damage, samples should then be placed within another plastic bag labeled with the study ID and study center ID. A bio-safety label should also be applied. Standard procedures for transporting biological samples as defined by the courier and in compliance with local regulations will be followed if different from recommended packaging instructions.

Sample Shipment.

IATA (International Air Transport Association) approved polystyrene transport boxes must be used.

For samples transported on wet ice:

The box should contain frozen ice blocks and protective packaging (polystyrene flocking), to allow for a minimum of 24 hours transport.

For samples transported on dry ice:

The box should contain dry-ice pellets (if pellets are not available then blocks may be used if protective packaging such as polystyrene flocking is included) to allow for a minimum of 72 hours transport.

Each package must be sealed in a cardboard box labeled with the courier airway bill.

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Clinical Pharmacology Study Protocol: Appendix A	
Drug substance:	AZD6140
Study Code:	D5130C05265
Appendix Edition No:	Final version 1.0
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Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A Randomized, Double-Blind, Two-Period Crossover Study to Assess Safety, Tolerability, and Pharmacokinetics Following Repeated Doses of AZD6140 (400 mg od) and Digoxin (0.25 mg od) in Healthy Male and Female Volunteers

I agree to the terms of this study protocol

AstraZeneca Clinical Development Team representative

Date
(Day Month Year)

ASTRAZENECA SIGNATURE(S)

A Randomized, Double-Blind, Two-Period Crossover Study to Assess Safety, Tolerability, and Pharmacokinetics Following Repeated Doses of AZD6140 (400 mg od) and Digoxin (0.25 mg od) in Healthy Male and Female Volunteers

I agree to the terms of this study protocol

AstraZeneca Research and Development site representative

Date
(Day Month Year)

ASTRAZENECA SIGNATURE(S)

A Randomized, Double-Blind, Two-Period Crossover Study to Assess Safety, Tolerability, and Pharmacokinetics Following Repeated Doses of AZD6140 (400 mg od) and Digoxin (0.25 mg od) in Healthy Male and Female Volunteers

I agree to the terms of this study protocol

AstraZeneca Research and Development site representative

Date
(Day Month Year)

STUDY NO.: D5130C05265
CENTER NO.: 001
DOC. CODE: 10
DATE TO IM. SRV.:

SIGNATURE OF PRINCIPAL INVESTIGATOR

A Randomized, Double-Blind, Two-Period Crossover Study to Assess Safety, Tolerability, and Pharmacokinetics Following Repeated Doses of AZD6140 (400 mg od) and Digoxin (0.25 mg od) in Healthy Male and Female Volunteers

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and local regulations.

Centre No.: 001

Signature:

Date
(Day Month Year)

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix B	
Drug substance:	AZD6140
Study Code:	D5130C05265
Appendix Edition No:	Final Version 1.0
Appendix Date:	

Appendix B
Additional Safety Information

1. DEFINITIONS OF ADVERSE EVENTS

Adverse event

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

Serious adverse event

A serious adverse event is an AE occurring during any study phase (eg, run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfils one or more of the following criteria:

- results in death
- is immediately life-threatening
- requires in-subject hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital abnormality or birth defect
- is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above?

The causality of SAEs (eg, their relationship to study treatment) will be assessed by the investigator(s), who in completing the relevant case report form must answer “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by the drug?” Further guidance on the definition of a SAE and a guide to the interpretation of the causality question is provided in this Appendix to the Clinical Pharmacology Study Protocol.

Other significant adverse event (OAE)

OAEs will be identified by the Drug Safety Physician and if applicable also by the Clinical Study Team Physician during the evaluation of safety data for the Clinical Study Report. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from study treatment, will be classified as OAEs. Examples of these are marked hematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment. For each OAE, a narrative may be written and included in

the Clinical Study Report.

2. FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

Life threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Out-patient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Examples of such events are:

- Angioedema not severe enough to require intubation but requiring iv. hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc.) or convulsions that do not result in hospitalisation
- Development of drug dependency or drug abuse

3. A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?

- Dechallenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Rechallenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a rechallenge.

- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

A "reasonable possibility" could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?

- Is there a known mechanism

Ambiguous cases should be considered as being a "reasonable possibility" of a causal relationship unless further evidence becomes available to refute this. Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

Clinical Pharmacology Study Protocol: Appendix C	
Drug substance:	AZD6140
Study Code:	D5130C05265
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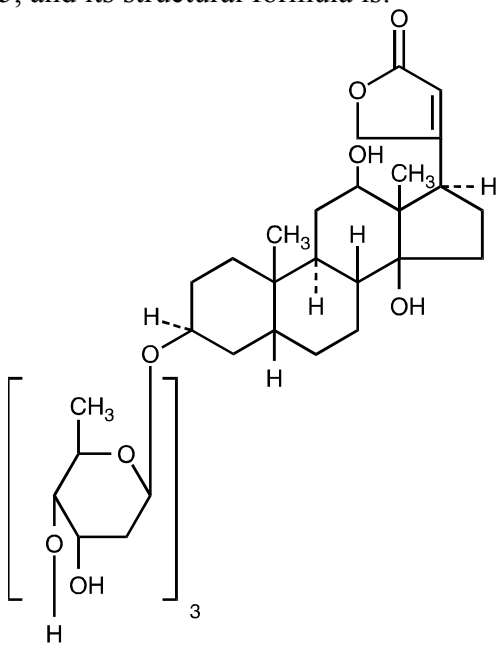
Appendix C
Digoxin Summary of Product Characteristics

PRODUCT INFORMATION

LANOXIN® (digoxin) Tablets, USP
125 mcg (0.125 mg) Scored I.D. Imprint Y3B (yellow)
250 mcg (0.25 mg) Scored I.D. Imprint X3A (white)

DESCRIPTION: LANOXIN (digoxin) is one of the cardiac (or digitalis) glycosides, a closely related group of drugs having in common specific effects on the myocardium. These drugs are found in a number of plants. Digoxin is extracted from the leaves of *Digitalis lanata*. The term "digitalis" is used to designate the whole group of glycosides. The glycosides are composed of two portions: a sugar and a cardenolide (hence "glycosides").

Digoxin is described chemically as (3β,5β,12β)-3-[(*O*-2,6-dideoxy-β-*D*-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-*D*-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-*D*-*ribo*-hexopyranosyl)oxy]-12,14-dihydroxy-card-20(22)-enolide. Its molecular formula is $C_{41}H_{64}O_{14}$, its molecular weight is 780.95, and its structural formula is:



Digoxin exists as odorless white crystals that melt with decomposition above 230°C. The drug is practically insoluble in water and in ether; slightly soluble in diluted (50%) alcohol and in chloroform; and freely soluble in pyridine.

LANOXIN is supplied as 125-mcg (0.125-mg) or 250-mcg (0.25-mg) tablets for oral administration. Each tablet contains the labeled amount of digoxin USP and the following inactive ingredients: corn and potato starches, lactose, and magnesium stearate. In addition, the dyes used in the 125-mcg (0.125-mg) tablets are D&C Yellow No. 10 and FD&C Yellow No. 6.

CLINICAL PHARMACOLOGY: Mechanism of Action: Digoxin inhibits sodium-potassium ATPase, an enzyme that regulates the quantity of sodium and potassium inside cells. Inhibition of the enzyme leads to an increase in the intracellular concentration of sodium and thus (by stimulation of sodium-calcium exchange) an increase in the intracellular concentration of calcium. The beneficial effects of digoxin result from direct actions on cardiac muscle, as well as

indirect actions on the cardiovascular system mediated by effects on the autonomic nervous system. The autonomic effects include: (1) a vagomimetic action, which is responsible for the effects of digoxin on the sinoatrial and atrioventricular (AV) nodes; and (2) baroreceptor sensitization, which results in increased afferent inhibitory activity and reduced activity of the sympathetic nervous system and renin-angiotensin system for any given increment in mean arterial pressure. The pharmacologic consequences of these direct and indirect effects are: (1) an increase in the force and velocity of myocardial systolic contraction (positive inotropic action); (2) a decrease in the degree of activation of the sympathetic nervous system and renin-angiotensin system (neurohormonal deactivating effect); and (3) slowing of the heart rate and decreased conduction velocity through the AV node (vagomimetic effect). The effects of digoxin in heart failure are mediated by its positive inotropic and neurohormonal deactivating effects, whereas the effects of the drug in atrial arrhythmias are related to its vagomimetic actions. In high doses, digoxin increases sympathetic outflow from the central nervous system (CNS). This increase in sympathetic activity may be an important factor in digitalis toxicity.

Pharmacokinetics: ***Absorption:*** Following oral administration, peak serum concentrations of digoxin occur at 1 to 3 hours. Absorption of digoxin from LANOXIN Tablets has been demonstrated to be 60% to 80% complete compared to an identical intravenous dose of digoxin (absolute bioavailability) or LANOXICAPS® (relative bioavailability). When LANOXIN Tablets are taken after meals, the rate of absorption is slowed, but the total amount of digoxin absorbed is usually unchanged. When taken with meals high in bran fiber, however, the amount absorbed from an oral dose may be reduced. Comparisons of the systemic availability and equivalent doses for oral preparations of LANOXIN are shown in Table 1.

Table 1: Comparisons of the Systemic Availability and Equivalent Doses for Oral Preparations of LANOXIN

Product	Absolute Bioavailability	Equivalent Doses (mcg)* Among Dosage Forms			
LANOXIN Tablets	60 - 80%	62.5	125	250	500
LANOXIN Elixir Pediatric	70 - 85%	62.5	125	250	500
LANOXICAPS®	90 - 100%	50	100	200	400
LANOXIN Injection/IV	100%	50	100	200	400

* For example, 125-mcg LANOXIN Tablets equivalent to 125-mcg LANOXIN Elixir Pediatric equivalent to 100-mcg LANOXICAPS equivalent to 100-mcg LANOXIN Injection/IV.

In some patients, orally administered digoxin is converted to inactive reduction products (e.g., dihydrodigoxin) by colonic bacteria in the gut. Data suggest that one in ten patients treated with digoxin tablets will degrade 40% or more of the ingested dose. As a result, certain antibiotics may increase the absorption of digoxin in such patients. Although inactivation of these bacteria by antibiotics is rapid, the serum digoxin concentration will rise at a rate consistent with the elimination half-life of digoxin. The magnitude of rise in serum digoxin concentration relates to the extent of bacterial inactivation, and may be as much as two-fold in some cases.

Distribution: Following drug administration, a 6- to 8-hour tissue distribution phase is observed. This is followed by a much more gradual decline in the serum concentration of the drug, which is dependent on the elimination of digoxin from the body. The peak height and slope of the early portion (absorption/distribution phases) of the serum concentration-time curve are

dependent upon the route of administration and the absorption characteristics of the formulation. Clinical evidence indicates that the early high serum concentrations do not reflect the concentration of digoxin at its site of action, but that with chronic use, the steady-state post-distribution serum concentrations are in equilibrium with tissue concentrations and correlate with pharmacologic effects. In individual patients, these post-distribution serum concentrations may be useful in evaluating therapeutic and toxic effects (see DOSAGE AND ADMINISTRATION: Serum Digoxin Concentrations).

Digoxin is concentrated in tissues and therefore has a large apparent volume of distribution. Digoxin crosses both the blood-brain barrier and the placenta. At delivery, the serum digoxin concentration in the newborn is similar to the serum concentration in the mother. Approximately 25% of digoxin in the plasma is bound to protein. Serum digoxin concentrations are not significantly altered by large changes in fat tissue weight, so that its distribution space correlates best with lean (i.e., ideal) body weight, not total body weight.

Metabolism: Only a small percentage (16%) of a dose of digoxin is metabolized. The end metabolites, which include 3 ß-digoxigenin, 3-keto-digoxigenin, and their glucuronide and sulfate conjugates, are polar in nature and are postulated to be formed via hydrolysis, oxidation, and conjugation. The metabolism of digoxin is not dependent upon the cytochrome P-450 system, and digoxin is not known to induce or inhibit the cytochrome P-450 system.

Excretion: Elimination of digoxin follows first-order kinetics (that is, the quantity of digoxin eliminated at any time is proportional to the total body content). Following intravenous administration to healthy volunteers, 50% to 70% of a digoxin dose is excreted unchanged in the urine. Renal excretion of digoxin is proportional to glomerular filtration rate and is largely independent of urine flow. In healthy volunteers with normal renal function, digoxin has a half-life of 1.5 to 2.0 days. The half-life in anuric patients is prolonged to 3.5 to 5 days. Digoxin is not effectively removed from the body by dialysis, exchange transfusion, or during cardiopulmonary bypass because most of the drug is bound to tissue and does not circulate in the blood.

Special Populations: Race differences in digoxin pharmacokinetics have not been formally studied. Because digoxin is primarily eliminated as unchanged drug via the kidney and because there are no important differences in creatinine clearance among races, pharmacokinetic differences due to race are not expected.

The clearance of digoxin can be primarily correlated with renal function as indicated by creatinine clearance. The Cockcroft and Gault formula for estimation of creatinine clearance includes age, body weight, and gender. Table 5 that provides the usual daily maintenance dose requirements of LANOXIN Tablets based on creatinine clearance (per 70 kg) is presented in the DOSAGE AND ADMINISTRATION section.

Plasma digoxin concentration profiles in patients with acute hepatitis generally fell within the range of profiles in a group of healthy subjects.

Pharmacodynamic and Clinical Effects: The times to onset of pharmacologic effect and to peak effect of preparations of LANOXIN are shown in Table 2.

Table 2: Times to Onset of Pharmacologic Effect and to Peak Effect of Preparations of LANOXIN

Product	Time to Onset of Effect*	Time to Peak Effect*
LANOXIN Tablets	0.5 - 2 hours	2 - 6 hours
LANOXIN Elixir Pediatric	0.5 - 2 hours	2 - 6 hours
LANOXICAPS	0.5 - 2 hours	2 - 6 hours
LANOXIN Injection/IV	5 - 30 minutes†	1 - 4 hours

* Documented for ventricular response rate in atrial fibrillation, inotropic effects and electrocardiographic changes.

† Depending upon rate of infusion.

Hemodynamic Effects: Digoxin produces hemodynamic improvement in patients with heart failure. Short- and long-term therapy with the drug increases cardiac output and lowers pulmonary artery pressure, pulmonary capillary wedge pressure, and systemic vascular resistance. These hemodynamic effects are accompanied by an increase in the left ventricular ejection fraction and a decrease in end-systolic and end-diastolic dimensions.

Chronic Heart Failure: Two 12-week, double-blind, placebo-controlled studies enrolled 178 (RADIANCE trial) and 88 (PROVED trial) patients with NYHA class II or III heart failure previously treated with digoxin, a diuretic, and an ACE inhibitor (RADIANCE only) and randomized them to placebo or treatment with LANOXIN. Both trials demonstrated better preservation of exercise capacity in patients randomized to LANOXIN. Continued treatment with LANOXIN reduced the risk of developing worsening heart failure, as evidenced by heart failure-related hospitalizations and emergency care and the need for concomitant heart failure therapy. The larger study also showed treatment-related benefits in NYHA class and patients' global assessment. In the smaller trial, these trended in favor of a treatment benefit.

The Digitalis Investigation Group (DIG) main trial was a multicenter, randomized, double-blind, placebo-controlled mortality study of 6801 patients with heart failure and left ventricular ejection fraction ≤0.45. At randomization, 67% were NYHA class I or II, 71% had heart failure of ischemic etiology, 44% had been receiving digoxin, and most were receiving concomitant ACE inhibitor (94%) and diuretic (82%). Patients were randomized to placebo or LANOXIN, the dose of which was adjusted for the patient's age, sex, lean body weight, and serum creatinine (see DOSAGE AND ADMINISTRATION), and followed for up to 58 months (median 37 months). The median daily dose prescribed was 0.25 mg. Overall all-cause mortality was 35% with no difference between groups (95% confidence limits for relative risk of 0.91 to 1.07). LANOXIN was associated with a 25% reduction in the number of hospitalizations for heart failure, a 28% reduction in the risk of a patient having at least one hospitalization for heart failure, and a 6.5% reduction in total hospitalizations (for any cause).

Use of LANOXIN was associated with a trend to increase time to all-cause death or hospitalization. The trend was evident in subgroups of patients with mild heart failure as well as more severe disease, as shown in Table 3. Although the effect on all-cause death or hospitalization was not statistically significant, much of the apparent benefit derived from effects on mortality and hospitalization attributed to heart failure.

Table 3: Subgroup Analyses of Mortality and Hospitalization During the First Two Years Following Randomization

		Risk of All-Cause Mortality or All-Cause Hospitalization*			Risk of HF-Related Mortality or HF-Related Hospitalization*		
	n	Placebo	LANOXIN	Relative risk†	Placebo	LANOXIN	Relative risk†
All patients (EF ≤0.45)	6801	604	593	0.94 (0.88-1.00)	294	217	0.69 (0.63-0.76)
NYHA I/II	4571	549	541	0.96 (0.89-1.04)	242	178	0.70 (0.62-0.80)
EF 0.25-0.45	4543	568	571	0.99 (0.91-1.07)	244	190	0.74 (0.66-0.84)
CTR ≤0.55	4455	561	563	0.98 (0.91-1.06)	239	180	0.71 (0.63-0.81)
NYHA III / IV	2224	719	696	0.88 (0.80-0.97)	402	295	0.65 (0.57-0.75)
EF <0.25	2258	677	637	0.84 (0.76-0.93)	394	270	0.61 (0.53-0.71)
CTR >0.55	2346	687	650	0.85 (0.77-0.94)	398	287	0.65 (0.57-0.75)
EF >0.45‡	987	571	585	1.04 (0.88-1.23)	179	136	0.72 (0.53-0.99)

* Number of patients with an event during the first 2 years per 1000 randomized patients.
† Relative risk (95% confidence interval).
‡ DIG Ancillary Study.

In situations where there is no statistically significant benefit of treatment evident from a trial's primary endpoint, results pertaining to a secondary endpoint should be interpreted cautiously.

Chronic Atrial Fibrillation: In patients with chronic atrial fibrillation, digoxin slows rapid ventricular response rate in a linear dose-response fashion from 0.25 to 0.75 mg/day. Digoxin should not be used for the treatment of multifocal atrial tachycardia.

INDICATIONS AND USAGE:

Heart Failure: LANOXIN is indicated for the treatment of mild to moderate heart failure. LANOXIN increases left ventricular ejection fraction and improves heart failure symptoms as evidenced by exercise capacity and heart failure-related hospitalizations and emergency care, while having no effect on mortality. Where possible, LANOXIN should be used with a diuretic and an angiotensin-converting enzyme inhibitor, but an optimal order for starting these three drugs cannot be specified.

Atrial Fibrillation: LANOXIN is indicated for the control of ventricular response rate in patients with chronic atrial fibrillation.

CONTRAINDICATIONS: Digitalis glycosides are contraindicated in patients with ventricular fibrillation or in patients with a known hypersensitivity to digoxin. A hypersensitivity reaction to other digitalis preparations usually constitutes a contraindication to digoxin.

WARNINGS:
Sinus Node Disease and AV Block: Because digoxin slows sinoatrial and AV conduction, the drug commonly prolongs the PR interval. The drug may cause severe sinus bradycardia or sinoatrial block in patients with pre-existing sinus node disease and may cause advanced or complete heart block in patients with pre-existing incomplete AV block. In such patients consideration should be given to the insertion of a pacemaker before treatment with digoxin.
Accessory AV Pathway (Wolff-Parkinson-White Syndrome): After intravenous digoxin therapy, some patients with paroxysmal atrial fibrillation or flutter and a coexisting accessory AV pathway have developed increased antegrade conduction across the accessory pathway bypassing the AV node, leading to a very rapid ventricular response or ventricular fibrillation. Unless conduction down the accessory pathway has been blocked (either pharmacologically or by surgery), digoxin should not be used in such patients. The treatment of paroxysmal supraventricular tachycardia in such patients is usually direct-current cardioversion.
Use in Patients with Preserved Left Ventricular Systolic Function: Patients with certain disorders involving heart failure associated with preserved left ventricular ejection fraction may be particularly susceptible to toxicity of the drug. Such disorders include restrictive cardiomyopathy, constrictive pericarditis, amyloid heart disease, and acute cor pulmonale. Patients with idiopathic hypertrophic subaortic stenosis may have worsening of the outflow obstruction due to the inotropic effects of digoxin.

PRECAUTIONS:
Use in Patients with Impaired Renal Function: Digoxin is primarily excreted by the kidneys; therefore, patients with impaired renal function require smaller than usual maintenance doses of digoxin (see DOSAGE AND ADMINISTRATION). Because of the prolonged elimination half-life, a longer period of time is required to achieve an initial or new steady-state serum concentration in patients with renal impairment than in patients with normal renal function. If appropriate care is not taken to reduce the dose of digoxin, such patients are at high risk for toxicity, and toxic effects will last longer in such patients than in patients with normal renal function.
Use in Patients with Electrolyte Disorders: In patients with hypokalemia or hypomagnesemia, toxicity may occur despite serum digoxin concentrations below 2.0 ng/mL, because potassium or magnesium depletion sensitizes the myocardium to digoxin. Therefore, it is desirable to maintain normal serum potassium and magnesium concentrations in patients being treated with digoxin. Deficiencies of these electrolytes may result from malnutrition, diarrhea, or prolonged vomiting, as well as the use of the following drugs or procedures: diuretics, amphotericin B, corticosteroids, antacids, dialysis, and mechanical suction of gastrointestinal secretions.

Hypercalcemia from any cause predisposes the patient to digitalis toxicity. Calcium, particularly when administered rapidly by the intravenous route, may produce serious arrhythmias in digitalized patients. On the other hand, hypocalcemia can nullify the effects of digoxin in humans; thus, digoxin may be ineffective until serum calcium is restored to normal.

These interactions are related to the fact that digoxin affects contractility and excitability of the heart in a manner similar to that of calcium.

Use in Thyroid Disorders and Hypermetabolic States: Hypothyroidism may reduce the requirements for digoxin. Heart failure and/or atrial arrhythmias resulting from hypermetabolic or hyperdynamic states (e.g., hyperthyroidism, hypoxia, or arteriovenous shunt) are best treated by addressing the underlying condition. Atrial arrhythmias associated with hypermetabolic states are particularly resistant to digoxin treatment. Care must be taken to avoid toxicity if digoxin is used.

Use in Patients with Acute Myocardial Infarction: Digoxin should be used with caution in patients with acute myocardial infarction. The use of inotropic drugs in some patients in this setting may result in undesirable increases in myocardial oxygen demand and ischemia.

Use During Electrical Cardioversion: It may be desirable to reduce the dose of digoxin for 1 to 2 days prior to electrical cardioversion of atrial fibrillation to avoid the induction of ventricular arrhythmias, but physicians must consider the consequences of increasing the ventricular response if digoxin is withdrawn. If digitalis toxicity is suspected, elective cardioversion should be delayed. If it is not prudent to delay cardioversion, the lowest possible energy level should be selected to avoid provoking ventricular arrhythmias.

Laboratory Test Monitoring: Patients receiving digoxin should have their serum electrolytes and renal function (serum creatinine concentrations) assessed periodically; the frequency of assessments will depend on the clinical setting. For discussion of serum digoxin concentrations, see DOSAGE AND ADMINISTRATION section.

Drug Interactions: Potassium-depleting *diuretics* are a major contributing factor to digitalis toxicity. *Calcium*, particularly if administered rapidly by the intravenous route, may produce serious arrhythmias in digitalized patients. *Quinidine, verapamil, amiodarone, propafenone, indomethacin*, *itraconazole*, *alprazolam,* and *spironolactone* raise the serum digoxin concentration due to a reduction in clearance and/or in volume of distribution of the drug, with the implication that digitalis intoxication may result. *Erythromycin* and *clarithromycin* (and possibly other *macrolide antibiotics*) and *tetracycline* may increase digoxin absorption in patients who inactivate digoxin by bacterial metabolism in the lower intestine, so that digitalis intoxication may result (see CLINICAL PHARMACOLOGY: Absorption). *Propantheline* and *diphenoxylate,* by decreasing gut motility, may increase digoxin absorption. *Antacids, kaolin-pectin, sulfasalazine, neomycin, cholestyramine,* certain *anticancer drugs*, and *metoclopramide* may interfere with intestinal digoxin absorption, resulting in unexpectedly low serum concentrations. *Rifampin* may decrease serum digoxin concentration, especially in patients with renal dysfunction, by increasing the non-renal clearance of digoxin. There have been inconsistent reports regarding the effects of other drugs [e.g., *quinine, penicillamine*] on serum digoxin concentration. *Thyroid* administration to a digitalized, hypothyroid patient may increase the dose requirement of digoxin. Concomitant use of digoxin and *sympathomimetics* increases the risk of cardiac arrhythmias. *Succinylcholine* may cause a sudden extrusion of potassium from muscle cells, and may thereby cause arrhythmias in digitalized patients. Although beta-adrenergic blockers or calcium channel blockers and digoxin may be useful in combination to control atrial fibrillation, their additive effects on AV node conduction can result in advanced or complete heart block.

Due to the considerable variability of these interactions, the dosage of digoxin should be individualized when patients receive these medications concurrently. Furthermore, caution

should be exercised when combining digoxin with any drug that may cause a significant deterioration in renal function, since a decline in glomerular filtration or tubular secretion may impair the excretion of digoxin.

Drug/Laboratory Test Interactions: The use of therapeutic doses of digoxin may cause prolongation of the PR interval and depression of the ST segment on the electrocardiogram. Digoxin may produce false positive ST-T changes on the electrocardiogram during exercise testing. These electrophysiologic effects reflect an expected effect of the drug and are not indicative of toxicity.

Carcinogenesis, Mutagenesis, Impairment of Fertility: There have been no long-term studies performed in animals to evaluate carcinogenic potential, nor have studies been conducted to assess the mutagenic potential of digoxin or its potential to affect fertility.

Pregnancy: ***Teratogenic Effects:*** Pregnancy Category C. Animal reproduction studies have not been conducted with digoxin. It is also not known whether digoxin can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. Digoxin should be given to a pregnant woman only if clearly needed.

Nursing Mothers: Studies have shown that digoxin concentrations in the mother's serum and milk are similar. However, the estimated exposure of a nursing infant to digoxin via breast feeding will be far below the usual infant maintenance dose. Therefore, this amount should have no pharmacologic effect upon the infant. Nevertheless, caution should be exercised when digoxin is administered to a nursing woman.

Pediatric Use: Newborn infants display considerable variability in their tolerance to digoxin. Premature and immature infants are particularly sensitive to the effects of digoxin, and the dosage of the drug must not only be reduced but must be individualized according to their degree of maturity. Digitalis glycosides can cause poisoning in children due to accidental ingestion.

Geriatric Use: The majority of clinical experience gained with digoxin has been in the elderly population. This experience has not identified differences in response or adverse effects between the elderly and younger patients. However, this drug is known to be substantially excreted by the kidney, and the risk of toxic reactions to this drug may be greater in patients with impaired renal function. Because elderly patients are more likely to have decreased renal function, care should be taken in dose selection, which should be based on renal function, and it may be useful to monitor renal function (see DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS: In general, the adverse reactions of digoxin are dose-dependent and occur at doses higher than those needed to achieve a therapeutic effect. Hence, adverse reactions are less common when digoxin is used within the recommended dose range or therapeutic serum concentration range and when there is careful attention to concurrent medications and conditions.

Because some patients may be particularly susceptible to side effects with digoxin, the dosage of the drug should always be selected carefully and adjusted as the clinical condition of the patient warrants. In the past, when high doses of digoxin were used and little attention was paid to clinical status or concurrent medications, adverse reactions to digoxin were more frequent and severe. Cardiac adverse reactions accounted for about one-half, gastrointestinal disturbances for about one-fourth, and CNS and other toxicity for about one-fourth of these adverse reactions. However, available evidence suggests that the incidence and severity of digoxin toxicity has decreased substantially in recent years. In recent controlled clinical trials, in patients with predominantly mild to moderate heart failure, the incidence of adverse experiences was

comparable in patients taking digoxin and in those taking placebo. In a large mortality trial, the incidence of hospitalization for suspected digoxin toxicity was 2% in patients taking LANOXIN compared to 0.9% in patients taking placebo. In this trial, the most common manifestations of digoxin toxicity included gastrointestinal and cardiac disturbances; CNS manifestations were less common.

Adults: ***Cardiac:*** Therapeutic doses of digoxin may cause heart block in patients with pre-existing sinoatrial or AV conduction disorders; heart block can be avoided by adjusting the dose of digoxin. Prophylactic use of a cardiac pacemaker may be considered if the risk of heart block is considered unacceptable. High doses of digoxin may produce a variety of rhythm disturbances, such as first-degree, second-degree (Wenckebach), or third-degree heart block (including asystole); atrial tachycardia with block; AV dissociation; accelerated junctional (nodal) rhythm; unifocal or multiform ventricular premature contractions (especially bigeminy or trigeminy); ventricular tachycardia; and ventricular fibrillation. Digoxin produces PR prolongation and ST segment depression which should not by themselves be considered digoxin toxicity. Cardiac toxicity can also occur at therapeutic doses in patients who have conditions which may alter their sensitivity to digoxin (see WARNINGS and PRECAUTIONS).

Gastrointestinal: Digoxin may cause anorexia, nausea, vomiting, and diarrhea. Rarely, the use of digoxin has been associated with abdominal pain, intestinal ischemia, and hemorrhagic necrosis of the intestines.

CNS: Digoxin can produce visual disturbances (blurred or yellow vision), headache, weakness, dizziness, apathy, confusion, and mental disturbances (such as anxiety, depression, delirium, and hallucination).

Other: Gynecomastia has been occasionally observed following the prolonged use of digoxin. Thrombocytopenia and maculopapular rash and other skin reactions have been rarely observed.

Table 4 summarizes the incidence of those adverse experiences listed above for patients treated with LANOXIN Tablets or placebo from two randomized, double-blind, placebo-controlled withdrawal trials. Patients in these trials were also receiving diuretics with or without angiotensin-converting enzyme inhibitors. These patients had been stable on digoxin, and were randomized to digoxin or placebo. The results shown in Table 4 reflect the experience in patients following dosage titration with the use of serum digoxin concentrations and careful follow-up. These adverse experiences are consistent with results from a large, placebo-controlled mortality trial (DIG trial) wherein over half the patients were not receiving digoxin prior to enrollment.

Table 4: Adverse Experiences In Two Parallel, Double-Blind, Placebo-Controlled Withdrawal Trials (Number of Patients Reporting)

Adverse Experience	Digoxin Patients (n = 123)	Placebo Patients (n = 125)
Cardiac		
Palpitation	1	4
Ventricular extrasystole	1	1
Tachycardia	2	1
Heart arrest	1	1
Gastrointestinal		
Anorexia	1	4
Nausea	4	2
Vomiting	2	1
Diarrhea	4	1
Abdominal pain	0	6
CNS		
Headache	4	4
Dizziness	6	5
Mental disturbances	5	1
Other		
Rash	2	1
Death	4	3

Infants and Children: The side effects of digoxin in infants and children differ from those seen in adults in several respects. Although digoxin may produce anorexia, nausea, vomiting, diarrhea, and CNS disturbances in young patients, these are rarely the initial symptoms of overdosage. Rather, the earliest and most frequent manifestation of excessive dosing with digoxin in infants and children is the appearance of cardiac arrhythmias, including sinus bradycardia. In children, the use of digoxin may produce any arrhythmia. The most common are conduction disturbances or supraventricular tachyarrhythmias, such as atrial tachycardia (with or without block) and junctional (nodal) tachycardia. Ventricular arrhythmias are less common. Sinus bradycardia may be a sign of impending digoxin intoxication, especially in infants, even in the absence of first-degree heart block. Any arrhythmia or alteration in cardiac conduction that develops in a child taking digoxin should be assumed to be caused by digoxin, until further evaluation proves otherwise.

OVERDOSAGE:

Treatment of Adverse Reactions Produced by Overdosage: Digoxin should be temporarily discontinued until the adverse reaction resolves. Every effort should also be made to correct factors that may contribute to the adverse reaction (such as electrolyte disturbances or concurrent medications). Once the adverse reaction has resolved, therapy with digoxin may be reinstituted, following a careful reassessment of dose.

Withdrawal of digoxin may be all that is required to treat the adverse reaction. However, when the primary manifestation of digoxin overdosage is a cardiac arrhythmia, additional therapy may be needed.

If the rhythm disturbance is a symptomatic bradyarrhythmia or heart block, consideration should be given to the reversal of toxicity with DIGIBIND® [Digoxin Immune Fab (Ovine)] (see Massive Digitalis Overdosage subsection), the use of atropine, or the insertion of a temporary cardiac pacemaker. However, asymptomatic bradycardia or heart block related to digoxin may require only temporary withdrawal of the drug and cardiac monitoring of the patient.

If the rhythm disturbance is a ventricular arrhythmia, consideration should be given to the correction of electrolyte disorders, particularly if hypokalemia (seeAdministration of Potassium subsection) or hypomagnesemia is present. DIGIBIND is a specific antidote for digoxin and may be used to reverse potentially life-threatening ventricular arrhythmias due to digoxin overdosage.

Administration of Potassium: Every effort should be made to maintain the serum potassium concentration between 4.0 and 5.5 mmol/L. Potassium is usually administered orally, but when correction of the arrhythmia is urgent and the serum potassium concentration is low, potassium may be administered cautiously by the intravenous route. The electrocardiogram should be monitored for any evidence of potassium toxicity (e.g., peaking of T waves) and to observe the effect on the arrhythmia. Potassium salts may be dangerous in patients who manifest bradycardia or heart block due to digoxin (unless primarily related to supraventricular tachycardia) and in the setting of massive digitalis overdosage (see Massive Digitalis Overdosage subsection).

Massive Digitalis Overdosage: Manifestations of life-threatening toxicity include ventricular tachycardia or ventricular fibrillation, or progressive bradyarrhythmias, or heart block. The administration of more than 10 mg of digoxin in a previously healthy adult, or more than 4 mg in a previously healthy child, or a steady-state serum concentration greater than 10 ng/mL often results in cardiac arrest.

DIGIBIND should be used to reverse the toxic effects of ingestion of a massive overdose. The decision to administer DIGIBIND to a patient who has ingested a massive dose of digoxin but who has not yet manifested life-threatening toxicity should depend on the likelihood that life-threatening toxicity will occur (see above).

Patients with massive digitalis ingestion should receive large doses of activated charcoal to prevent absorption and bind digoxin in the gut during enteroenteric recirculation. Emesis or gastric lavage may be indicated especially if ingestion has occurred within 30 minutes of the patient's presentation at the hospital. Emesis should not be induced in patients who are obtunded. If a patient presents more than 2 hours after ingestion or already has toxic manifestations, it may be unsafe to induce vomiting or attempt passage of a gastric tube, because such maneuvers may induce an acute vagal episode that can worsen digitalis-related arrhythmias.

Severe digitalis intoxication can cause a massive shift of potassium from inside to outside the cell, leading to life-threatening hyperkalemia. The administration of potassium supplements in the setting of massive intoxication may be hazardous and should be avoided. Hyperkalemia caused by massive digitalis toxicity is best treated with DIGIBIND; initial treatment with glucose and insulin may also be required if hyperkalemia itself is acutely life-threatening.

DOSAGE AND ADMINISTRATION:

General: Recommended dosages of digoxin may require considerable modification because of individual sensitivity of the patient to the drug, the presence of associated conditions, or the use

of concurrent medications. In selecting a dose of digoxin, the following factors must be considered:

1. The body weight of the patient. Doses should be calculated based upon lean (i.e., ideal) body weight.
2. The patient's renal function, preferably evaluated on the basis of estimated creatinine clearance.
3. The patient's age. Infants and children require different doses of digoxin than adults. Also, advanced age may be indicative of diminished renal function even in patients with normal serum creatinine concentration (i.e., below 1.5 mg/dL).
4. Concomitant disease states, concurrent medications, or other factors likely to alter the pharmacokinetic or pharmacodynamic profile of digoxin (see PRECAUTIONS).

Serum Digoxin Concentrations: In general, the dose of digoxin used should be determined on clinical grounds. However, measurement of serum digoxin concentrations can be helpful to the clinician in determining the adequacy of digoxin therapy and in assigning certain probabilities to the likelihood of digoxin intoxication. About two-thirds of adults considered adequately digitalized (without evidence of toxicity) have serum digoxin concentrations ranging from 0.8 to 2.0 ng/mL. However, digoxin may produce clinical benefits even at serum concentrations below this range. About two-thirds of adult patients with clinical toxicity have serum digoxin concentrations greater than 2.0 ng/mL. However, since one-third of patients with clinical toxicity have concentrations less than 2.0 ng/mL, values below 2.0 ng/mL do not rule out the possibility that a certain sign or symptom is related to digoxin therapy. Rarely, there are patients who are unable to tolerate digoxin at serum concentrations below 0.8 ng/mL. Consequently, the serum concentration of digoxin should always be interpreted in the overall clinical context, and an isolated measurement should not be used alone as the basis for increasing or decreasing the dose of the drug.

To allow adequate time for equilibration of digoxin between serum and tissue, sampling of serum concentrations should be done just before the next scheduled dose of the drug. If this is not possible, sampling should be done at least 6 to 8 hours after the last dose, regardless of the route of administration or the formulation used. On a once-daily dosing schedule, the concentration of digoxin will be 10% to 25% lower when sampled at 24 versus 8 hours, depending upon the patient's renal function. On a twice-daily dosing schedule, there will be only minor differences in serum digoxin concentrations whether sampling is done at 8 or 12 hours after a dose.

If a discrepancy exists between the reported serum concentration and the observed clinical response, the clinician should consider the following possibilities:

1. Analytical problems in the assay procedure.
2. Inappropriate serum sampling time.
3. Administration of a digitalis glycoside other than digoxin.
4. Conditions (described in WARNINGS and PRECAUTIONS) causing an alteration in the sensitivity of the patient to digoxin.
5. Serum digoxin concentration may decrease acutely during periods of exercise without any associated change in clinical efficacy due to increased binding of digoxin to skeletal muscle.

Heart Failure: ***Adults:*** Digitalization may be accomplished by either of two general approaches that vary in dosage and frequency of administration, but reach the same endpoint in terms of total amount of digoxin accumulated in the body.

1. If rapid digitalization is considered medically appropriate, it may be achieved by administering a loading dose based upon projected peak digoxin body stores. Maintenance dose can be calculated as a percentage of the loading dose.
2. More gradual digitalization may be obtained by beginning an appropriate maintenance dose, thus allowing digoxin body stores to accumulate slowly. Steady-state serum digoxin concentrations will be achieved in approximately five half-lives of the drug for the individual patient. Depending upon the patient's renal function, this will take between 1 and 3 weeks.

Rapid Digitalization with a Loading Dose: Peak digoxin body stores of 8 to 12 mcg/kg should provide therapeutic effect with minimum risk of toxicity in most patients with heart failure and normal sinus rhythm. Because of altered digoxin distribution and elimination, projected peak body stores for patients with renal insufficiency should be conservative (i.e., 6 to 10 mcg/kg) [see PRECAUTIONS].

The loading dose should be administered in several portions, with roughly half the total given as the first dose. Additional fractions of this planned total dose may be given at 6- to 8-hour intervals, **with careful assessment of clinical response before each additional dose.**

If the patient's clinical response necessitates a change from the calculated loading dose of digoxin, then calculation of the maintenance dose should be based upon the amount actually given.

A single initial dose of 500 to 750 mcg (0.5 to 0.75 mg) of LANOXIN Tablets usually produces a detectable effect in 0.5 to 2 hours that becomes maximal in 2 to 6 hours. Additional doses of 125 to 375 mcg (0.125 to 0.375 mg) may be given cautiously at 6- to 8-hour intervals until clinical evidence of an adequate effect is noted. The usual amount of LANOXIN Tablets that a 70-kg patient requires to achieve 8 to 12 mcg/kg peak body stores is 750 to 1250 mcg (0.75 to 1.25 mg).

LANOXIN Injection is frequently used to achieve rapid digitalization, with conversion to LANOXIN Tablets or LANOXICAPS for maintenance therapy. If patients are switched from intravenous to oral digoxin formulations, allowances must be made for differences in bioavailability when calculating maintenance dosages (see Table 1, CLINICAL PHARMACOLOGY).

Maintenance Dosing: The doses of digoxin used in controlled trials in patients with heart failure have ranged from 125 to 500 mcg (0.125 to 0.5 mg) once daily. In these studies, the digoxin dose has been generally titrated according to the patient's age, lean body weight, and renal function. Therapy is generally initiated at a dose of 250 mcg (0.25 mg) once daily in patients under age 70 with good renal function, at a dose of 125 mcg (0.125 mg) once daily in patients over age 70 or with impaired renal function, and at a dose of 62.5 mcg (0.0625 mg) in patients with marked renal impairment. Doses may be increased every 2 weeks according to clinical response.

In a subset of approximately 1800 patients enrolled in the DIG trial (wherein dosing was based on an algorithm similar to that in Table 5) the mean (± SD) serum digoxin concentrations at 1 month and 12 months were 1.01 ± 0.47 ng/mL and 0.97 ± 0.43 ng/mL, respectively.

The maintenance dose should be based upon the percentage of the peak body stores lost each day through elimination. The following formula has had wide clinical use:

Maintenance Dose = Peak Body Stores (i.e., Loading Dose) x % Daily Loss/100

Where: % Daily Loss = 14 + Ccr/5
(Ccr is creatinine clearance, corrected to 70 kg body weight or 1.73 m^2 body surface area.)

Table 5 provides average daily maintenance dose requirements of LANOXIN Tablets for patients with heart failure based upon lean body weight and renal function:

Table 5: Usual Daily Maintenance Dose Requirements (mcg) of LANOXIN for Estimated Peak Body Stores of 10 mcg/kg

Corrected Ccr (mL/min per 70 kg)*	Lean Body Weight kg	50	60	70	80	90	100	Number of Days Before Steady State Achieved†
	lb	110	132	154	176	198	220	
0		62.5‡	125	125	125	187.5	187.5	22
10		125	125	125	187.5	187.5	187.5	19
20		125	125	187.5	187.5	187.5	250	16
30		125	187.5	187.5	187.5	250	250	14
40		125	187.5	187.5	250	250	250	13
50		187.5	187.5	250	250	250	250	12
60		187.5	187.5	250	250	250	375	11
70		187.5	250	250	250	250	375	10
80		187.5	250	250	250	375	375	9
90		187.5	250	250	250	375	500	8
100		250	250	250	375	375	500	7

*Ccr is creatinine clearance, corrected to 70 kg body weight or 1.73 m^2 body surface area. *For adults,* if only serum creatinine concentrations (Scr) are available, a Ccr (corrected to 70 kg body weight) may be estimated in men as (140 - Age)/Scr. For women, this result should be multiplied by 0.85. *Note:* This equation cannot be used for estimating creatinine clearance in infants or children.

†If no loading dose administered.

‡62.5 mcg = 0.0625 mg

Example: Based on Table 5, a patient in heart failure with an estimated lean body weight of 70 kg and a Ccr of 60 mL/min should be given a dose of 250 mcg (0.25 mg) daily of LANOXIN Tablets, usually taken after the morning meal. If no loading dose is administered, steady-state serum concentrations in this patient should be anticipated at approximately 11 days.

Infants and Children: In general, divided daily dosing is recommended for infants and young children (under age 10). In the newborn period, renal clearance of digoxin is diminished and suitable dosage adjustments must be observed. This is especially pronounced in the premature infant. Beyond the immediate newborn period, children generally require proportionally larger doses than adults on the basis of body weight or body surface area. Children over 10 years of age require adult dosages in proportion to their body weight. Some researchers have suggested that infants and young children tolerate slightly higher serum concentrations than do adults.

Daily maintenance doses for each age group are given in Table 6 and should provide therapeutic effects with minimum risk of toxicity in most patients with heart failure and normal sinus rhythm. These recommendations assume the presence of normal renal function:

Table 6: Daily Maintenance Doses in Children with Normal Renal Function

Age	Daily Maintenance Dose (mcg/kg)
2 to 5 Years	10 to 15
5 to 10 Years	7 to 10
Over 10 Years	3 to 5

In children with renal disease, digoxin must be carefully titrated based upon clinical response.

It cannot be overemphasized that both the adult and pediatric dosage guidelines provided are based upon average patient response and substantial individual variation can be expected. Accordingly, ultimate dosage selection must be based upon clinical assessment of the patient.

Atrial Fibrillation: Peak digoxin body stores larger than the 8 to 12 mcg/kg required for most patients with heart failure and normal sinus rhythm have been used for control of ventricular rate in patients with atrial fibrillation. Doses of digoxin used for the treatment of chronic atrial fibrillation should be titrated to the minimum dose that achieves the desired ventricular rate control without causing undesirable side effects. Data are not available to establish the appropriate resting or exercise target rates that should be achieved.

Dosage Adjustment When Changing Preparations: The difference in bioavailability between LANOXIN Injection or LANOXICAPS and LANOXIN Elixir Pediatric or LANOXIN Tablets must be considered when changing patients from one dosage form to another.

Doses of 100 mcg (0.1 mg) and 200 mcg (0.2 mg) of LANOXICAPS are approximately equivalent to 125-mcg (0.125-mg) and 250-mcg (0.25-mg) doses of LANOXIN Tablets and Elixir Pediatric, respectively (see Table 1 in CLINICAL PHARMACOLOGY: Pharmacokinetics).

HOW SUPPLIED:

LANOXIN (digoxin) Tablets, Scored 125 mcg (0.125 mg): Bottles of 100 with child-resistant cap (NDC 0173-0242-55) and 1000 (NDC 0173-0242-75); unit dose pack of 100 (NDC 0173-0242-56). Imprinted with LANOXIN and Y3B (yellow).

Store at 25°C (77°F); excursions permitted to 15 to 30°C (59 to 86°F) [see USP Controlled Room Temperature] in a dry place and protect from light.

LANOXIN (digoxin) Tablets, Scored 250 mcg (0.25 mg): Bottles of 100 with child-resistant cap (NDC 0173-0249-55), 1000 (NDC 0173-0249-75), and 5000 (NDC 0173-0249-80); unit dose pack of 100 (NDC 0173-0249-56). Imprinted with LANOXIN and X3A (white).

Store at 25°C (77°F); excursions permitted to 15 to 30°C (59 to 86°F) [see USP Controlled Room Temperature] in a dry place.

GlaxoSmithKline
Research Triangle Park, NC 27709

RL-972

AstraZeneca

Clinical Pharmacology Study Protocol: Appendix D	
Drug substance:	AZD6140
Study Code:	D5130C05265
Appendix Edition No:	Final Version 1.0
Appendix Date:	

Appendix D
Instructions for Collection, Storage and Transport of Blood Samples for Genetic Analysis

1. BLOOD SAMPLE COLLECTION

Ideally, blood should be collected into ***9/10 ml polypropylene tubes*** containing the ***anticoagulant EDTA***. Recommended tubes are detailed in the table below. After collection, blood tubes must be gently ***inverted*** several times to ensure thorough mixing of EDTA with the sample to prevent clotting.

Table of recommended blood tubes for genotyping sample collection

Polypropylene Collection Tube	Part #	Comments
	1066 US 1066.001 UK	SARSTEDT Monovette® EDTA KE - 9ml
	368457 USA/UK	Becton-Dickinson Vacutainer™ K2E - 10ml
	455036 USA/UK	Greiner Bio-one Vacuette® K3E EDTA K3 - 9ml

- ***Glass tubes MUST NOT be used*** as they may break during transport and freeze-thaw cycles.
- ***Heparin MUST NOT be used as an anticoagulant*** as it may interfere with downstream genotyping methodology.

The collection tubes must be labeled with the following information:

- Unique sample ID (compliant with protocol)
- Study ID (and Study Center ID, if available)
- Date of sample collection.

2. STORAGE AT THE STUDY CENTER AND TRANSPORT

After collection, blood samples must be stored appropriately at the site of collection and transported to the Central Handling Facility, or Designated DNA Processing Laboratory, ***as soon as possible***. The table below shows guidelines for sample storage and transport:

Table to show the recommended storage conditions for blood samples immediately after collection

Option	***Storage Temperature at Study Center***	***Maximum Duration***	***Transport Temperature***	***Delivery Time***
1	+ 4°C (fridge)	24 hours	0 - 4°C (ice bricks)	24 hours
2	+ 4°C (fridge)	24 hours	Less than -20°C (dry ice)	24-72 hours
3	-20°C (freezer) or -70°C	Up to 1 month	Less than -20°C (dry ice)	24-72 hours

- **IF BLOOD SAMPLES ARE TO BE STORED AT -20°C OR LESS, NON-FROST FREE FREEZERS MUST BE USED TO PREVENT REPEATED FREEZE-THAW OF BLOOD WHICH MAY REDUCE YIELD & QUALITY OF THE DNA OBTAINED.**

- **SAMPLES MUST NOT BE THAWED AND THEN RE-FROZEN AT ANY POINT**

The Central Handling Facility, or Designated DNA Processing Laboratory, must be notified of the shipment of any samples prior to dispatch. Ideally, the dispatch note must be sent by either fax or email and must contain the following information:

- Study ID, number of samples and list of sample ID's
- Courier name, airway bill number and date of shipment
- Shipment condition (wet ice or dry ice)
- Contact name and address

Considerations should be made to ensure that the samples are delivered during working hours and within 24-72 hours of dispatch.

3. RECOMMENDED PACKAGING INSTRUCTIONS

For safety reasons, all blood samples must be contained. Samples should be individually placed in a clip-lock bag labeled with the sample ID and sealed. Samples may then be batched and again sealed within a second clip-lock bag labeled with the study ID. For ease of further packaging and protection from damage, samples should then be placed within another plastic bag labeled with the study ID and study center ID. A bio-safety label should also be applied. Standard procedures for transporting biological samples as defined by the courier and in compliance with local regulations will be followed if different from recommended packaging instructions.

Sample Shipment.

IATA (International Air Transport Association) approved polystyrene transport boxes must be used.

For samples transported on wet ice:

The box should contain frozen ice blocks and protective packaging (polystyrene flocking), to allow for a minimum of 24 hours transport.

For samples transported on dry ice:

The box should contain dry-ice pellets (if pellets are not available then blocks may be used if protective packaging such as polystyrene flocking is included) to allow for a minimum of 72 hours transport.

Each package must be sealed in a cardboard box labeled with the courier airway bill.