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Introduction

In this study, we provide an investigational approach that reuses clinical trial data. We used the Vivli clinical research data sharing platform to determine whether digoxin could improve survival outcomes in patients with metastatic renal cell carcinoma (mRCC) who are treated with tyrosine kinase inhibitors (TKIs). Data of 12 completed clinical trials investigating tyrosine kinase inhibitors in patients with mRCC were collected from this data sharing platform. Individual participant data from all these studies were accessible, which were further utilized to gather data on survival endpoints (progression-free survival and overall survival) as well as relevant covariates.

In this report, we present our hypothesis on the potential effect of digoxin in mRCC.

Background

Renal cell carcinoma (RCC) is the most common type of renal cancer. Renal cell carcinomas can be classified into 4 different stages (I to IV). In stages I to III the disease is localized to the kidney, while in stage IV the disease is locally advanced or metastasized. 25-30% of patients present with advanced disease (stage IV). Stage IV carcinomas cannot be cured by resecting the tumor and hence require further systemic treatment. Although several improvements have been made in the treatment of RCC, survival outcomes for stage IV patients in particular remain poor.

RCC is highly resistant to common chemotherapies. Historically systemic treatment mainly consisted of immunotherapy using cytokines (interleukin-2, interferon-alfa). With the identification of angiogenesis as an important factor in the underlying pathophysiology of RCC, the first targeted angiogenesis inhibitors were developed. These include tyrosine kinase inhibitors (TKIs), mammalian target of rapamycin (mTOR) inhibitors (everolimus, temsirolimus) and bevacizumab (monoclonal antibody). The first TKIs turned out to greatly improve survival outcomes in RCC patients compared to cytokine therapy, and were approved in the 2000s by the FDA and EMA.

TKIs reduce angiogenesis by blocking vascular endothelial growth factor (VEGF) receptors and platelet derived growth factor (PDGF) receptors, inhibiting the blood flow toward tumor tissue and lowering the oxygen supply. However, a large proportion of tumors eventually become resistant to TKI treatment. It is thought that hypoxia inducible factors (HIFs) play an essential role in how tumors adapt to hypoxic environments. HIFs are transcription factors that upregulate VEGF and PDGF among others, decreasing the efficacy of TKI treatment.

The identification of the upregulation of HIFs (particularly HIF1-alpha) as an important mechanism in which tumors adapt to hypoxic environments led to drug repurposing studies. A screening of approximately 3000 drugs by Zhang et al. found that cardiac glycosides, among which digoxin, could inhibit the synthesis of HIF1-alpha *in vitro*. This effect was reversible however, as digoxin washout resulted in HIF levels returning to prior levels after 24 hours.

Consequently these agents were investigated further in mice models with transplanted tumors, where the administration of digoxin showed a decrease in tumor growth.

Another important finding was the increase in overall survival in retrospective analyses in cohorts consisting of breast cancer, colon cancer, head and neck cancer and hepatocellular cancer, when patients were using digoxin during the time the underwent chemotherapy (5 year survival rate of 65% vs. 52% respectively). This finding however is not yet reported for renal cell carcinomas.

Considering the outcomes in RCC treated with TKIs are still relatively poor, and a plausible mechanism by which digoxin can have a synergistic effect on this via hypoxia resistant pathways, this study was performed. It was hypothesized that the use of digoxin alongside TKIs could increase progression-free survival (PFS) and overall survival (OS) in these patients. This was investigated using retrospective data of 12 clinical trials where RCC patients were treated with a TKI (sunitinib, sorafenib or axitinib).

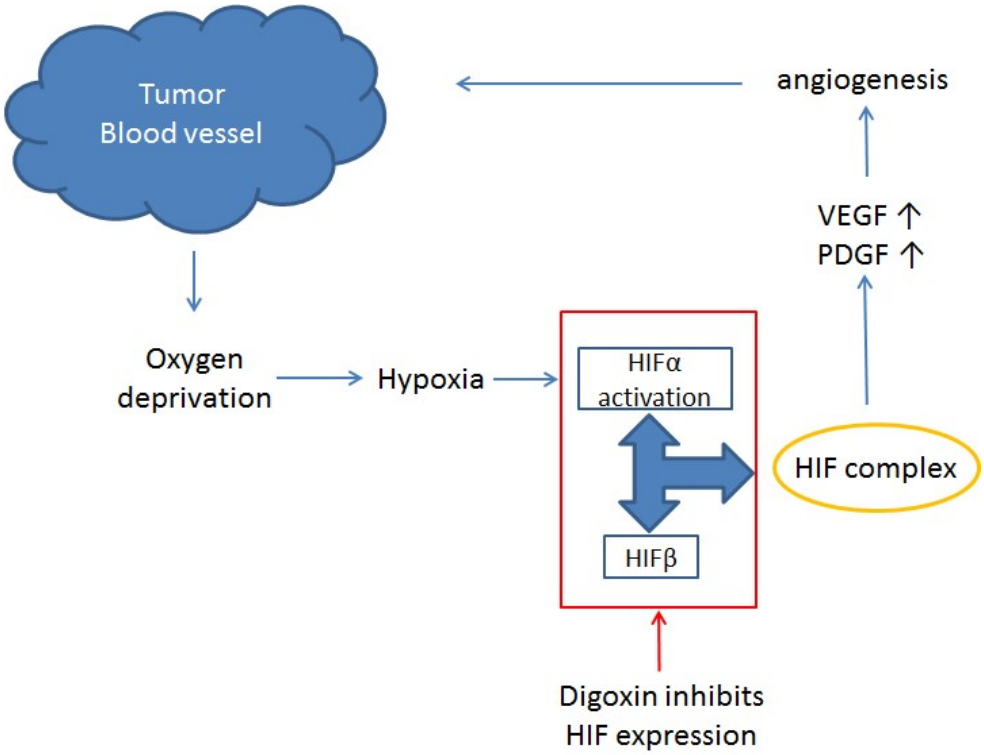

Figure 2, digoxin inhibits HIF expression. Our hypothesis is that when tumor become resistant for TKI treatment by upregulating VEGF via HIF, digoxin is able to reverse this mechanism.

Materials and methods

Study population

Patients in 12 trials who were all diagnosed with metastatic or advanced (i.e. stage IV) renal cell carcinoma (mRCC). The drugs investigated were axitinib, sorafenib and sunitinib (TKIs), temsirolimus (mTOR inhibitor) and interferon alpha. Primary outcomes in these 12 studies were PFS or objective response rate (ORR). Secondary outcomes were diverse, but in all studies included measurements of both PFS and OS.

In 4 of the 12 trials the subjects received first-line systemic therapy, 6 of the 12 trials second-line therapy, and in 2 trials both first-line and second-line therapy were studied. All but one trial excluded mRCC patients with a non-clearcell subtype. Subjects that were included mostly had mRCC with clearcell histology. For full details see supplementary table S1.

Study design

This is a retrospective cohort study with digoxin as exposure variable. Patient data of 12 clinical trials were extracted from the Vivli Center for Global Clinical Research Data database.

Outcomes of interest in our study are progression-free survival (PFS) and overall survival (OS). To account for possible confounding variables, data on factors known to be of prognostic value were collected. These include: randomized treatment arm, 1stline or 2ndline systemic therapy, ECOG Performance Status, IMDC risk factors and MSKCC risk factors. Basic clinical and demographic factors such as age, gender, ethnicity, nationality, body mass index, mRCC subtype (clearcell or non-clearcell), prior nephrectomy and prior radiation therapy were collected as well.

To guide clinical decision makers in the selection for systemic therapy for each patient, there are risk stratification models available. For the purposes of this study, these models can be used to adjust the survival outcomes as it is an important prognostic factor. At the time of conducting these 12 studies, the model that was used were the MSKCC criteria. However, this model predates the time when there were any angiogenesis inhibitors available. This model has since been updated by the Heng et al. , which is the one that is currently used (IMDC model). As this model is the one that is validated based on angiogenesis targeted therapy, it was decided to use this model rather than the old MSKCC criteria. The only difference is that the IMDC model does not take LDH into account, but it was decided to include LDH into our analysis separately.

To assess the independent effect of digoxin on PFS and OS, crude and adjusted hazard ratios were calculated using a Cox proportional hazards model. Significance levels were determined using the likelihood ratio statistic. A likelihood ratio test was performed where a p-value of <0.05 was considered statistically significant.

Inclusion criteria

Patients who were digoxin users during the time they were included in one of these trials were extracted. Patients were defined to be a ‘digoxin user’ when they were taking digoxin at least >50% during the time they underwent systemic therapy with a TKI. Based on the available literature, no persisting effect of digoxin on HIF is to be expected. Because of limited data, it was decided not to put a threshold on digoxin dose.

Exclusion criteria

If our hypothesis is correct there is no synergistic effect of digoxin to be expected together with cytokine therapy or mTOR inhibitor. Therefore, patients who received interferon-alpha or temsirolimus in these studies were excluded.

Data collection

Individual patient data of all included subjects were collected. These include time data to determine survival (PFS and OS) as well as covariates known and/or suspected to be of prognostic value (for complete list see below). To correct for possible heterogeneity in outcome measurement in the 12 trials, start date of PFS and OS was set to be the randomization data (some of the trials used date of first dose of intervention drug as the start date).

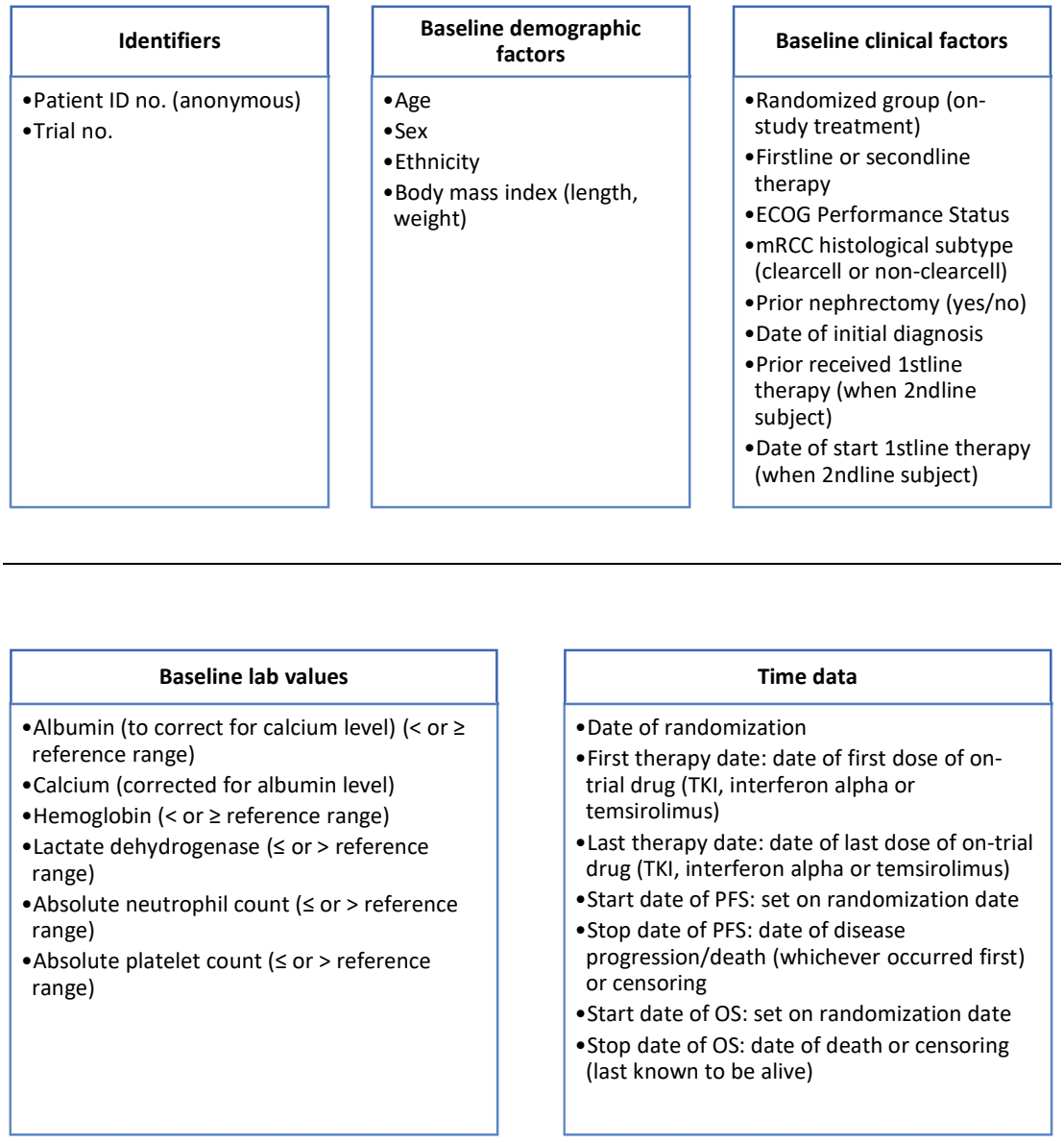

Figure 3, list of collected variables used for regression analysis.

Calculated values
•1. Time interval between initial diagnosis: date first dose 1stline therapy - date initial diagnosis •2. Corrected calcium level: calcium level + 0.8 x (40 – albumin level in g/l) •3. Body mass index: weight (in kg) – height^2 (in metres) •4. IMDC risk factors group (good: 0 risk factors; intermediate: 1-2 risk factors; poor: ≥3 risk factors) • - Time interval diagnosis to 1stline treatment <1 year • - Karnofsky Performance Status <80% (=ECOG Performance Status ≥2) [23] • - Elevated corrected calcium level • - Decreased hemoglobin level • - Elevated neutrophil count • - Elevated platelet count •5. PFS: date of disease progression/death/censoring – randomization date •6. OS: date of death/censoring – randomization date

Figure 4, list of collected variables used for regression analysis.

Statistical analysis

Cox proportional hazards model

Multivariate regression was performed with a cox proportional hazards model. To exclude covariates without prognostic value a backwards selection strategy was used (include all covariates measured >90%, then exclude covariates that are insignificant). To determine whether a covariate was a significant contributor for PFS/OS, likelihood ratio tests were performed (alpha level 0.05). With this a final prognostic model (only covariates of significant influence on PFS/OS) was generated and tested against digoxin use as an offset variable.

To mitigate the problem of the small digoxin group, a two-step procedure was employed. First, a fox model was used to compute a risk score for the selected covariates on the total sample without digoxin as the exposure variable. Subsequently, a second cox model was fitted using only the risk score and digoxin as covariates. In this way, adjusted hazard ratios for digoxin use could be calculated [34]. The software that was used was Rstudio (2017, version 3.4.1.).

Steps

1. Include all subjects in the model
2. Fit a cox proportional hazards function (without digoxin) on covariates collected, backwards selection (p=0.05 threshold)
3. Calculate risk score (PFS and OS) for each subject using this function ('predict' function in R)
4. Fit a 2nd cox model with input risk score + digoxin
5. Calculate adjusted hazard ratio (& significance level)

Outcomes of interest

Progression free survival	Overall survival
• Crude hazard ratio (95% confidence interval, significance level) • Adjusted hazard ratio (95% confidence interval, significance level)	• Crude hazard ratio (95% confidence interval, significance level) • Adjusted hazard ratio (95% confidence interval, significance level)

Results

Digoxin users were included in only 2 of the 12 trials, the other 10 trials were excluded from our analysis. Because of the low number of digoxin users it was decided to pool these 2 trials together as 1 cohort rather than to perform a meta-analysis.

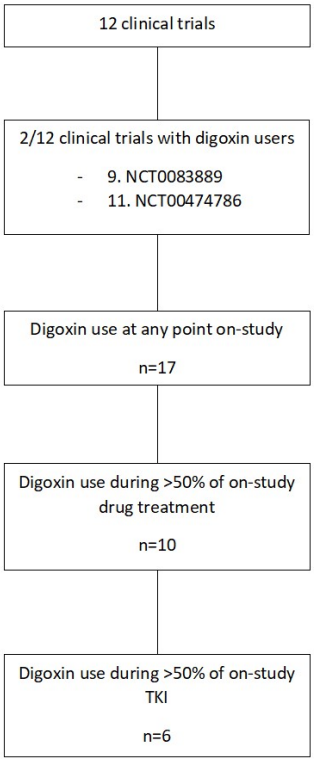

Figure 5, flowchart of digoxin cases selection.

Trial no.	Drug(s) tested	Population	Study arm(s)	Sample size	Year(s) inclusion	Primary endpoint
NCT00083889	Sunitinib, interferon alpha	1stline	1. Sunitinib 2. Interferon alpha	1. n=375 2. n=375	2004-2005	Progression free survival
NCT00474786	Sorafenib, temsirolimus	2ndline, after sunitinib	1. Sorafenib 2. Temsirolimus	1. n=253 2. n=259	2007-2011	Progression free survival
Total	Sunitinib, Sorafenib, Interferon alpha, Temsirolimus	1stline & 2ndline after sunitinib therapy	Diverse	All: n=1262 TKI only: n=628	2003-2011	Progression free survival

Table 1, description of all trials included in this analysis. For the full set of included trials, see supplementary table S1.

Digoxin users

Of all the 12 clinical trials only 2 trials had included subjects with concomitant digoxin use, namely NCT00083889 and NCT00474786. In total, 17 subjects had used digoxin at any time point on-study. Out of these 17, 10 subjects had used digoxin during at least 50% of the time they were treated with the on-study drug. All of these subjects were using digoxin during the full period they were treated with the on-study drug in the trial (i.e. 100% of the time). The other 7 subjects were excluded in our analysis, since no significant effect of digoxin is to be expected in these short time spans. For full details, see table 2.

Of all the 10 continuous digoxin users, 6 were randomized to receive treatment with a TKI, 2 to interferon alpha and 2 to temsirolimus. According to our hypothesis there is no synergistic effect to be expected with digoxin in combination with cytokines or mTOR inhibitors, so these subjects were excluded from our analysis. Exploratory analyses with these groups included were however performed.

The 6 digoxin users of interest were included in the NCT00083889 and NCT00474786 trials between July 20, 2005 and January 14, 2011. Indications among these 6 were: atrial fibrillation (3), atrial flutter (1), hypertension (1) (most likely also atrial fibrillation, see table concomitant medication) and unknown (1). Most common other medications of these 6 subjects were: antihypertensive drugs (5), cholesterol-lowering drugs (4), antithrombotic agents (4), analgesics (3), beta-blockers (2), antiarrhythmic drugs (2) and proton pump inhibitors (2). For full details see the supplementary table S4 in the supplementary appendix.

Baseline demographic and clinical characteristics

All 6 digoxin users were male and Caucasian, compared to 73% male and 84% Caucasian in the control group. Digoxin users were between 46 and 76 years old (median: 69 years); controls were between 21 and 87 years old (median: 61 years). BMI for digoxin users were between 23.5 and 51.5 (median: 31.0); for the controls the BMI was between 17.1 and 66.2 (median: 26.7).

2 digoxin users were randomized to the sunitinib arm in the NCT00083889 (sunitinib vs. interferon alpha) study in 1stline treatment; 4 digoxin users were randomized to the sorafenib arm in the NCT00474786 (sorafenib vs. temsirolimus) study in 2ndline treatment after sunitinib. 4 digoxin users were diagnosed with histological confirmed clearcell mRCC (67%), while the other 2 had non-clearcell histology (33%), of which 1 was of the papillary subtype. This compares to 86% of controls who had clearcell mRCC. Prior treatments digoxin users underwent were nephrectomy in 5 cases (83%), and radiation therapy in 2 cases (33%). Controls underwent nephrectomy and/or radiation therapy in 88% and 17% of cases respectively. Interesting was that the time interval from initial diagnosis to slightly longer for digoxin compared to controls (median: 56 vs. 41 weeks respectively).

According to the IMDC risk stratification model, 3 digoxin users had good risk disease (50%, 2 intermediate risk disease (33%) and 1 poor risk disease (17%). If the risk is stratified according to the old MSKCC model the same results are generated, with the only difference that 2 digoxin users switch their status between intermediate and poor risk disease.

Of the controls 27% had good risk, 61% intermediate risk, and 12% poor risk disease according to IMDC criteria. When applying the MSKCC criteria instead, 26% had good risk, 63% intermediate risk and 11% poor risk disease. IMDC and MSKCC risk group could not be determined in 12 and 13 cases respectively (both 2%), because of missing data.

Digoxin group vs. control group among subjects randomized to receive TKI

Variable	Digoxin users n=6	Controls n=621
Study arm – no. (%)		
○ Sunitinib (1stline)	2 (33%)	373 (60%)
○ Sorafenib (2ndline)	4 (67%)	248 (40%)
Sex – no. (%)		
○ Male	6 (100%)	452 (73%)
○ Female	-	169 (27%)
Age – median (range)	69 (46-76)	61 (21-87)
Ethnicity – no. (%)		*9 unknown
○ White	6 (100%)	512 (84%)
○ Asian	-	57 (9%)
○ Black	-	4 (1%)
○ Other	-	39 (6%)
Body mass index – median (range)	31.0 (23.5-51.5)	26.7 (17.1-66.2)
Histological subtype – no. (%)		
○ Clear cell	4 (67%)	536 (86%)
○ Non clear cell	2 (33%)	85 (14%)
Prior nephrectomy – no. (%)	5 (83%)	547 (88%)
Prior radiation therapy – no. (%)	2 (33%)	108 (17%)
Time diagnosis to treatment <1 year – no. (%)	2 (33%)	341 (55%)
Time diagnosis to treatment (weeks) – median (range)	56 (12-319)	41 (0-1279)
ECOG Performance Status – no. (%)		
○ 0	1 (17%)	347 (56%)
○ 1	5 (83%)	274 (44%)
IMDC risk factors – no. (%)		* 12 missing
○ 0 – Good	3 (50%)	167 (27%)
○ 1-2 – Intermediate	2 (33%)	370 (61%)
○ >2 - Poor	1 (17%)	72 (12%)
MSKCC risk factors – no. (%)		* 13 missing
○ 0 – Good	3 (50%)	159 (26%)
○ 1-2 – Intermediate	2 (33%)	380 (63%)
○ >2 - Poor	1 (17%)	69 (11%)
Baseline albumin level <lower limit normal – no. (%)	-	*4 missing 75 (12%)
Baseline lactate dehydrogenase >upper limit normal – no. (%)	2 (33%)	*4 missing 99 (16%)

Table 2, baseline characteristics of included digoxin users and controls.

Cox proportional hazards models

Cox model for progression free survival

In the first cox model, progression free survival was significantly affected by the following 5 covariates:

Covariate	p-level
Treatment arm (sunitinib 1stline or sorafenib 2ndline)	0.000
ECOG Performance Status (0 or 1)	0.016
Albumin level (<normal)	0.001
Lactate dehydrogenase level (>normal)	0.037
IMDC risk group (good, intermediate, poor)	0.000

Table 3, covariates significantly predicting progression free survival.

Other covariates with no significant predicting effect were omitted from the model. This cox model was used as the input for generating a 'predict' function. The effect of digoxin use was determined by fitting a second cox model with the predict function + digoxin as inputs.

Cox model for overall survival

The same was done for overall survival as secondary endpoint. On overall survival, 4 covariates had a significant effect in the cox model:

Covariate	p-level
ECOG Performance Status (0 or 1)	0.000
Albumin level (<normal)	0.000
Lactate dehydrogenase level (>normal)	0.000
IMDC risk group (good, intermediate, poor)	0.000

Table 4, covariates significantly predicting progression free survival.

And again other covariates with no significant predicting effect were omitted from the model. This includes atrial fibrillation as indication (coefficient 1.162; p=0.62). And also this cox model was used as the input for generating a 'predict' function. The effect of digoxin use was determined by fitting a second cox model with the predict function + digoxin as inputs.

Outcomes

Using the approach with two cox models generated the following results:

Digoxin use (under TKI treatment)	Progression free survival	p-value	Overall survival	p-value
Crude hazard ratio	1.255 (95%CI 0.468-3.363)	0.651	1.114 * (95% CI 0.416-2.988)	0.830
Adjusted hazard ratio	1.221 (95% CI 0.455-3.274)	0.691	0.691 (95% CI 0.257-1.859)	0.464

Table 5, crude and adjusted hazard ratios comparing digoxin use and controls on progression free survival and overall survival.

*crossing survival curves

Between the two groups, no significant differences in survival outcomes were found. Crude hazard ratios for digoxin use were 1.255 (95%CI 0.468-3.363; p=0.651) for PFS and 1.114 (95%CI 0.416-2.988; p=0.691) for OS. For OS however survival curves were crossing, so strictly put a hazard ratio cannot be interpreted as such here. Significance levels were calculated using the log-rank test.

Adjusted hazard ratios for digoxin use, using the cox models as mentioned, were 1.221 (95% CI 0.455-3.274; p=0.691) for PFS and 0.691 (95% CI 0.257-1.859; p=0.464) for OS. Significance levels were calculated using the log-rank test.

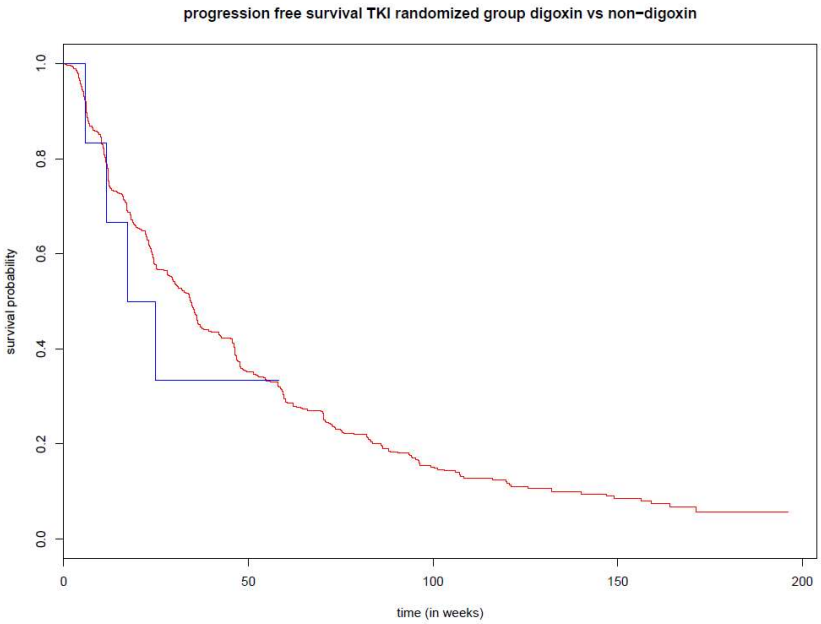

Figure 6, progression free survival curves of digoxin (blue) vs. controls (red).

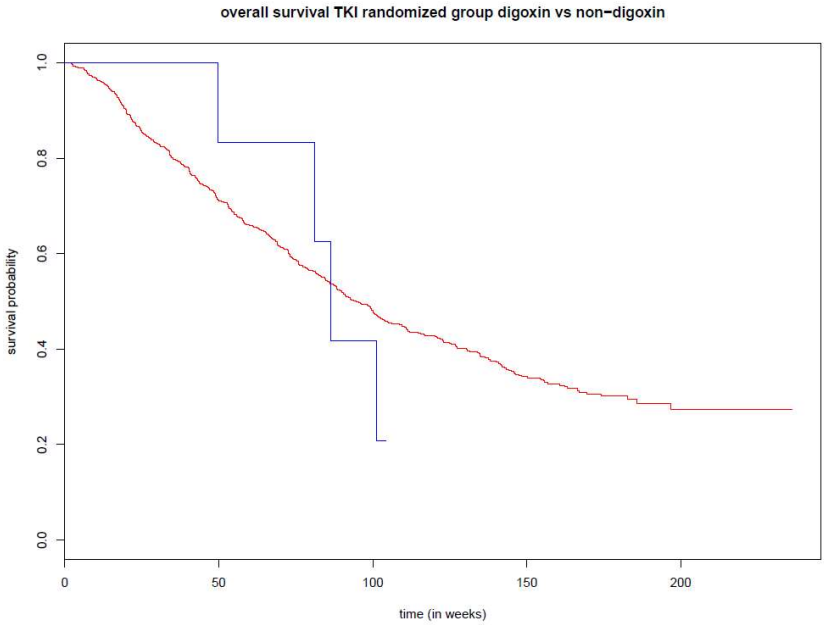

Figure 7, overall survival curves of digoxin (blue) vs. controls (red).