Association patient characteristics on fludarabine exposure in patients with hematopoietic allogenic stem cell transplantation

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ABSTRACT

Background

Hematopoietic allogenic stem cell transplantation provides a potential curative treatment for patients suffering from malignant or non-malignant diseases. Conditioning by fludarabine and busulfan forms an essential step during this procedure. Fludarabine is currently dosed based on body surface area. A retrospective study from Langenhorst showed association of fludarabine exposure with event outcomes in patients after allogenic SCT. In contrast, a RCT TARGET study did not find significant differences in between classical and TDM dosing of fludarabine. It was partially hypothesized that this discrepancy was related towards applied inclusion criteria of TARGET study.

Aim

This study evaluated whether patient characteristics applied as inclusion criteria of TARGET study explain the difference in between retrospective study of Langenhorst and TARGET study. Therefore, main aim of this research was to study the association of TARGET inclusion criteria towards fludarabine AUC attainment.

Method

A retrospective study was conducted whereby criteria from RCT TARGET study were applied on the patient population from the study of Langenhorst. Patients complied to all criteria were placed in cohort named “included” and who did not in “excluded”. These cohorts were compared on the following outcomes:

Primary outcome: Fludarabine AUC attainment

Secondary outcome: 2-year Event Free Survival Probability after Allogenic stem cell transplantation.

Furthermore, a multivariate logistic and stepwise regression analysis was performed to find patient characteristics that were associated towards target attainment.

Main results

For AUC attainment it was found that rate of underexposure in between included (n=93) and excluded (n=99) was significantly different. For optimal and above optimal exposure difference was insignificant.

For event-free-survival, overexposure showed a significantly increased event-risk in comparison to optimal exposure (HR 3.355) within excluded cohort. Within included cohort this was insignificant. Suboptimal exposure risk on events within included and excluded was insignificant towards optimal exposure within both cohorts.

Multivariate logistics regression analysis showed for children, multiple myeloma and auto-immune disease and moderate kidney function to be significantly associated towards target attainment. In addition, from stepwise regression analysis underlying disease, age category (kids or adults) and renal function were included as predictors for target attainment.

Discussion and conclusion

This study showed association of patient characteristics applied as criteria from TARGET study on fludarabine AUC attainment. Comparison of both cohorts based on event-free-survival remains however explorative. Further follow-up research including sufficient patients for all characteristics should be considered to evaluate the effect of characteristics on event outcomes.

Introduction

Hematopoietic allogenic stem cell transplantation (SCT) is a therapeutic intervention providing a potential curative treatment for multiple hematological diseases. Indications for SCT vary from malignant to non-malignant disorders [1]. To eradicate malignant cells and suppress immune system, conditioning by chemotherapeutic agents prior allogenic SCT forms an essential step before transferring hematopoietic stem cells (figure 1) [2]. In general, conditioning is performed over 4 consecutive days prior an allogenic HCT. A regular used conditioning regimen consists of alkylating agents fludarabine and busulfan [3,4].

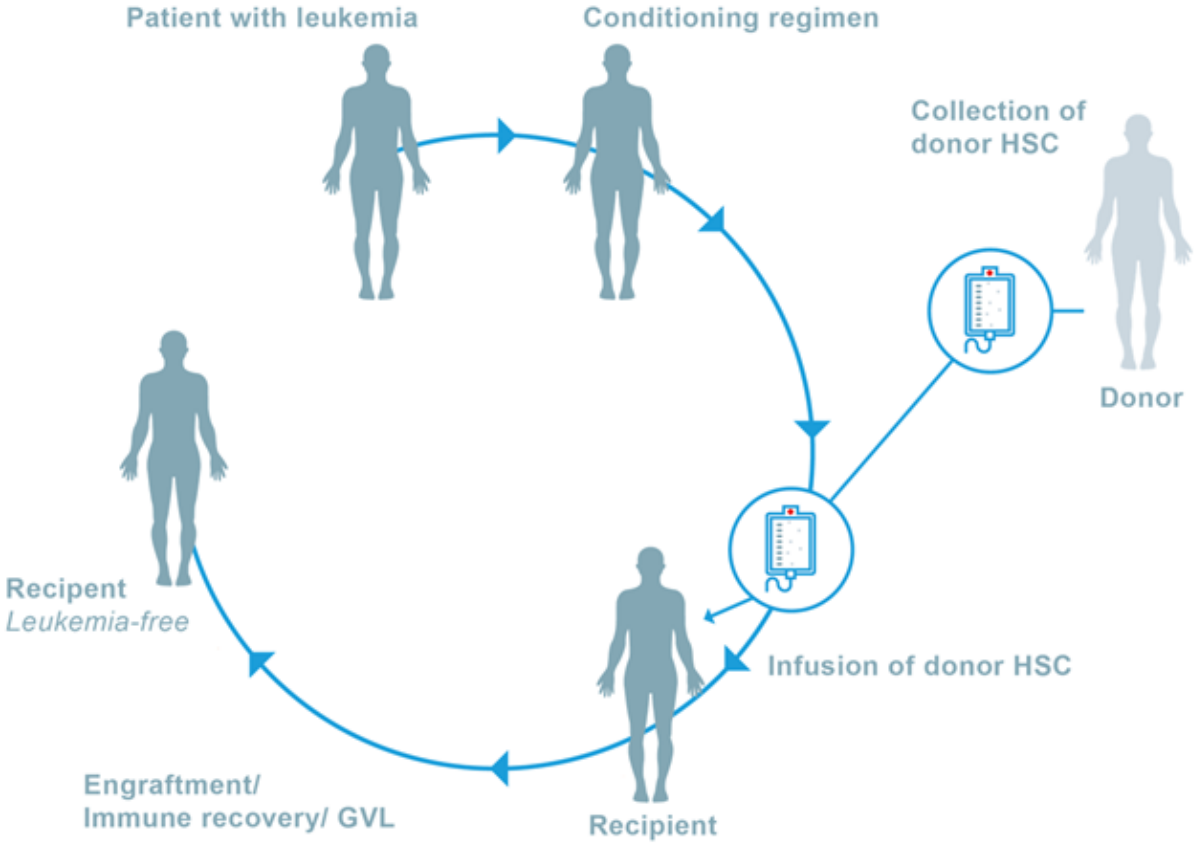

Figure 1 Schematic Display Hematopoietic Allogenic SCT [from Priothera[1]]

Fludarabine is administered as prodrug fludarabine-monophosphate. After uptake it is converted into 2-fludarabine-Ara-A (2-F-Ara). 2-F-Ara can be quantified from blood samples as this form is extracellular located and intracellular metabolites are hardly detectable. After intracellular uptake 2-F-Ara is converted into active fludarabine-ATP and induces apoptosis [5]. The pharmacological mechanism is inhibition of DNA synthesis. Clearance of fludarabine is mainly done by kidneys [5,6].

Due evidenced correlation of busulfan exposure with events like graft-failures, transplantation-related-mortality and acute toxicity, therapeutic drug monitoring (TDM) during busulfan conditioning is nowadays routinely applied. [7,8]. In contrast, TDM for fludarabine is not applied prior to allogenic SCT. Potential use of fludarabine TDM was driven by a retrospective study (n=192) from Langenhorst et al. This study showed association of fludarabine exposure with event outcomes in patients after

[1] From Hematopoietic Cell Transplantation | Priothera [Internet]. Priothera.com. 2023 [cited 2024 Oct 3]. Available from: https://priothera.com/hematopoietic-cell-transplantation/

undergoing allogenic- SCT. This involved events like relapse, non-relapse mortality (NRM) or graft failure. It was suggested that fludarabine exposure could predict event free survival (EFS) after SCT. Therefore, it was hypothesized that TDM based dosing of fludarabine could be relevant in daily practice [9,10]. In contrast, outcomes of a randomized clinical trial (RCT) named "TARGET" did not support this [11]. In this study a BSA dosed cohort was compared with another cohort whereby an optimal exposure range of 15-25 mg*h/l was targeted by TDM. This range was derived from the retrospective study of Langenhorst et al. [9,11]. Still, no significant differences in outcomes were found. The difference in between finding from retrospective and clinical TARGET study could have several explanations. First, both populations of the studies were not similar in patient characteristics. In contrast to retrospective study of Langenhorst, TARGET study applied inclusion criteria whereby children, certain underlying diseases or renal function below 40 ml/min were excluded.

As inclusion criteria are used to create an eligible study population for RCT TARGET study based on patient characteristics, the knowledge gab is into what extent patient characteristics determine association of fludarabine exposure with clinical outcomes. Furthermore, it is questioned whether inclusion criteria explain the discrepancy in outcome from both studies. Therefore, the purpose of this study is to evaluate to what extent patient characteristics as renal function, age and underlying disease influence fludarabine exposure and target attainment in patients undergoing allogenic-SCT.

Methods

Study design and population

A retrospective study was conducted with available PK-PD data of patients undergoing an allogenic-SCT between 2010 and 2017 at University Medical Center Utrecht [11]. As an early informed consent was previously asked for use of personal data from the study of Langenhorst, no extra consent was required.

The patient population underwent myeloablative conditioning by receiving a chemotherapy regime with a cumulative dose of 160 mg/m^2 fludarabine and busulfan with a cumulative AUC of 90 mg*h/l. Regimen consisted of first 1-hour intravenous infusion of fludarabine-monophosphate over 4 consecutive days from -5 day to -2 day prior SCT. After administration, a 3-hour intravenous infusion of busulfan was given.

Sampling

Sampling of fludarabine was performed in an earlier PK/PD study [8]. First blood samples were collected on day 1,2 and if available on day 4 according to busulfan TDM protocol. As these samples were originally meant for busulfan AUC determination, samples were taken 4,5,6, and 7 hours after fludarabine administration. Additional samples were available taken 15-45 minutes after fludarabine administration in patients who underwent allogenic-SCT since 2016. [cited directly from Langenhorst et al. 2019[2]]. Fludarabine metabolite F-Ara-A was quantified via Liquid Chromatography-Mass spectrometry (LCMS). An earlier developed PK/PD model was used to estimate fludarabine AUC [8]. This was done using measured concentrations of fludarabine as dependent variables in an earlier PK-PD model [8].

Outcomes of interest

Cumulative fludarabine AUC attainment was used as primary outcome of interest. This was done as for other outcomes such as Event-Free-Survival (EFS) could not be powered based on number of patients per cohort in this study.

To visualize distributions of AUC attainment, the following categories were defined as “fludarabine AUC groups” derived from the retrospective study [8,9]:

- suboptimal exposure with an AUC range < 15 mg*h/l
- optimal exposure with an AUC range of 15-25 mg*h/l
- overexposure with an AUC > 25 mg*h/l.

Secondary outcome was event free-survival probability within two years after allogenic SCT. Data of events were available as these were earlier prospectively collected and registered in a time-to-event model (Langenhorst et al) [10]. Events were defined as relapse, graft failure and non-relapse-mortality.

Procedures

Comparison cohorts

To evaluate whether patient characteristics were associated with fludarabine exposure and to test whether inclusion criteria explain the contrast in between retrospective study and TARGET, criteria of the TARGET trial were applied on the study population from retrospective study [11]. Therefore, patients were stratified into two cohorts based on established criteria from the protocol of TARGET

[2] Langenhorst J, Charlotte van Kesteren, Erik, Thomas, Nierkens S, Lindemans CA, et al. Fludarabine exposure in the conditioning prior to allogeneic hematopoietic cell transplantation predicts outcomes. Blood Advances. 2019 Jul 19;3(14):2179–87.

study. Patient population complied to all criteria were named “included cohort”, whereas patients who did not comply to all criteria were named “excluded cohort”. The criteria were as follows:

- patients aged 18 years or older at date of transplantation
- patients diagnosed with underlying disease except: Bone marrow (BM) failure, Immunodeficiency, auto-immune disease, childhood malignancy, multiple myeloma, plasma cell leukemia and metabolic inborn errors.
- patients with an eGFR renal function of 40 ml/min or higher

Statistical analysis

A chi-square test was performed to evaluate significant differences in between exposure attainment from both included and excluded cohorts. Furthermore, the EFS outcome from the original dataset was used to compare AUC groups of both cohort studies by using a cox proportional hazard model, adjusted for potential confounders as age, renal function, underlying diseases. Lastly, a multivariable logistic regression analysis was made on the original dataset to test whether RCT criteria related patient characteristics were associated with fludarabine exposure and optimal target attainment. The outcome was binominal defined as total cumulative fludarabine AUC within or out target range of 15-25 mg*h/l. In addition, a forward inclusion and backward deletion was used with significance value of 0.05 and removal of 0.1. The following variables were used in the analysis: age (categorial: kids under 18 years, adults above 18 years), renal function (categorial: Low <50 ml/min , moderate 50-90 ml/min and good >90 ml/min) and underlying disease. To calculate odds ratio, subgroup within the categorical variable with highest number of patients was taken as reference. For statistical analysis, R version 4.2.2 was used with the following packages: survival, survminer, cowplot2, ggplot2, gridextra, olsrr, patchwork and dplyr.

Results

Patient population

192 patients were available from the retrospective study and further subdivided into two cohorts of 93 and 99 patients named “included” and “excluded” respectively. Patient characteristics are depicted in Table 1.

Table 1 Patient Characteristics: MDS: Myelodysplastic syndrome

	Total population (n=192)	**Included (n=93)**	**Excluded (n=99)**
Gender			
Males	115 (59.9%)	55 (59.13%)	60 (60.6%)
Females	77 (40.1%)	38 (40.9%)	39 (39.4%)
Mean Age (years) at SCT	35.0	49.4	21.5
Children (under 18 years at SCT)	68	0	68
Adults (18 years and older at SCT)	124	93	31
Mean Renal function (ml/min)	110.0 (sd 26.2)	109.7 (sd 24.9)	110.3 (sd 27.4)
Mean Body Surface Area (BSA) (m^2)	1.58	1.91	1.26
Mean Weight (kg)	60.4	76.5	45.3
Underlying Disease			
Acute Leukemia	45 (23.4)	45 (48.4)	0
Auto-immune disease	4 (2.08)	0	4 (4.04)
Bone marrow failure	10 (5.21)	0	10 (10.1)
Childhood malignancy	7 (3.65)	0	7 (7.07)
Chronic Leukemia	3 (1.56)	3 (3.23)	0
Immunedeficiency	27 (14.1)	0	27 (27.3)
Low- High Risk MDS	30 (9.90)	29 (31.2)	1 (1.01)
Lymphoma	16 (8.33)	16 (17.2)	0
Metabolic/inborn errors	27 (14.1)	0	27 (27.3)
Multiple Myeloma	21 (10.9)	0	21 (21.2)
Plasma cell leukemia	2 (1.04)	0	2 (2.02)

Fludarabine AUC Attainment

Fludarabine AUC distributions over the cohorts are depicted in figure 2. Red, green and blue colored histograms represent respectively suboptimal, optimal and above optimal exposure of fludarabine. Statistical description of AUC distributions is depicted in table 2. Rate of underexposure was significantly different in between included and excluded. For optimal and above optimal differences in between both cohorts were insignificant.

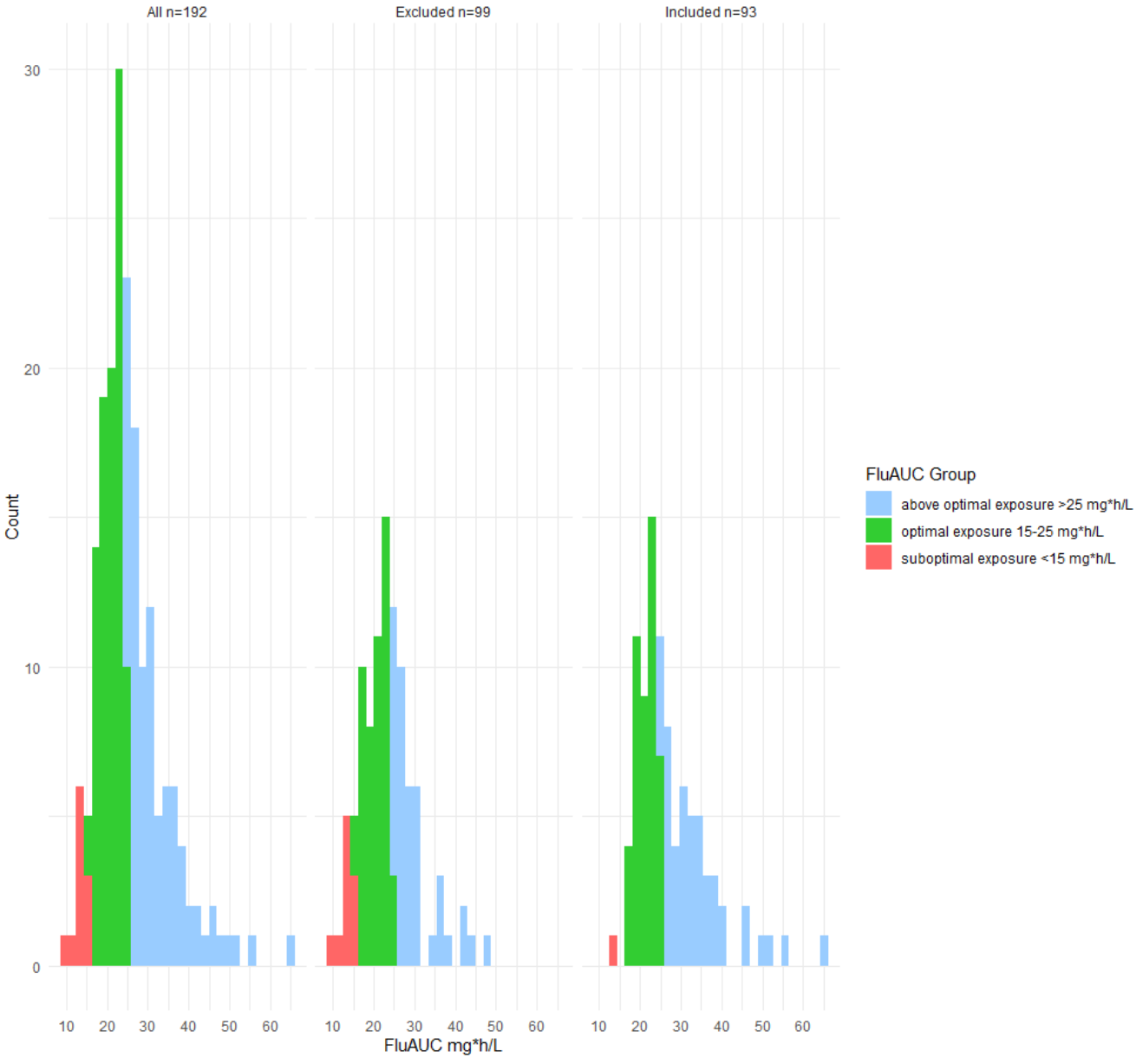

Figure 2 Histogram of Fludarabine AUC attainment in total population, excluded cohort and Included cohort

Table 2 Statistical AUC description of the patient cohorts

	Total Patient population	**Excluded**	**Included**	**Chi^2test Unadjusted p-value included vs excluded**
Patients n=	192	99	93	
Mean AUC Fludarabine (mg*h/l)	25.63	23.52	27.67	
Suboptimal (%)	5.73	10.10	1.08	0.017*
Optimal exposed (%)	49.48	49.47	49.46	0.999
Above optimal (%)	44.79	40.40	49.46	0.264
Standard deviation AUC sd	8.27	7.00	9.03	
Variance AUC (s^2)	68.39	49.10	81.47	

2 year-Event-Free-Survival (EFS)

Figure 3 depicts adjusted EFS probability curves of both cohorts each categorized by fludarabine exposure group. Figure 3a depicts EFS curves of the optimal exposures from both included and excluded cohorts. Event risk for included cohort appeared to be insignificant in comparison to excluded cohort. As shown in figure 3b, excluded optimal exposure range showed a significantly lower risk on events in comparison to overexposure (*p = 0.007)*. Within included cohort, adjusted event risk in between optimal and overexposure was insignificant (*p=0.072)*. For both cohorts, suboptimal exposure was not significantly associated with risk on events towards optimal exposure.

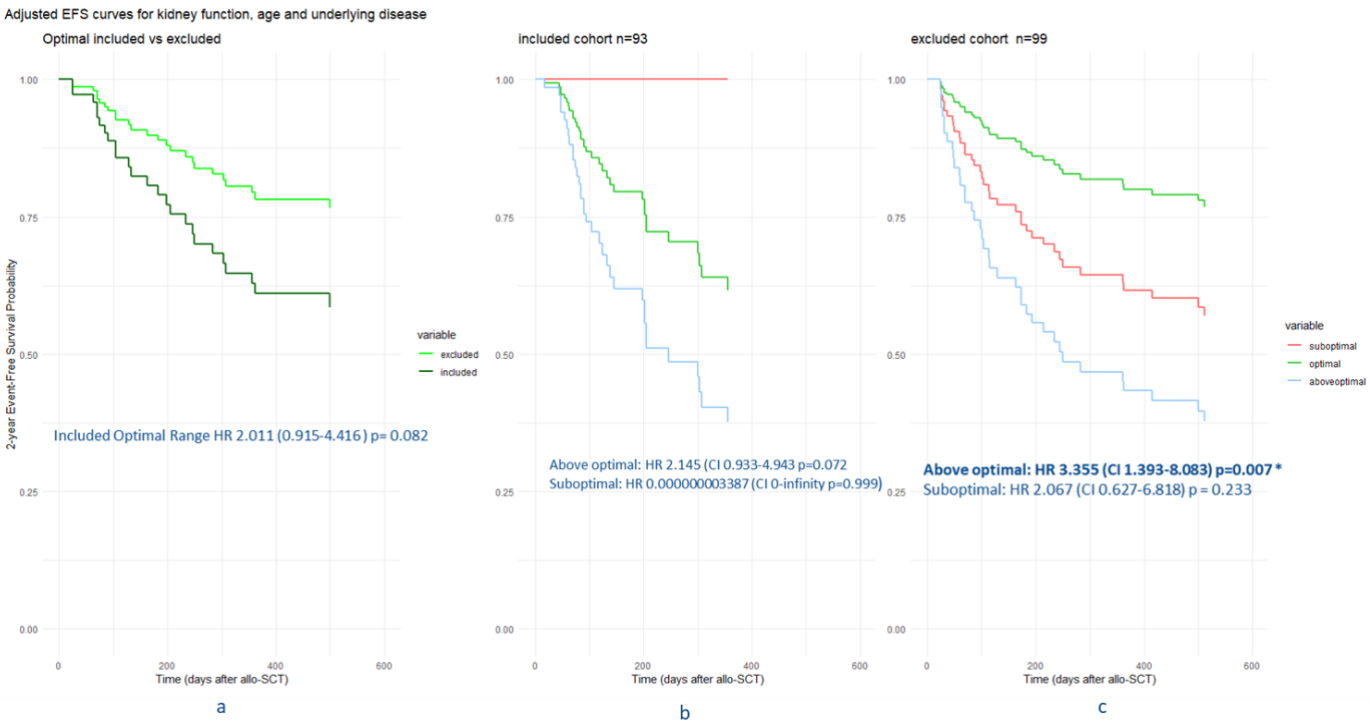

Figure 3. a. EFS curves optimal exposure from included and excluded cohorts plotted from COX-hazard model b. EFS Probability curves per FluAUC group adjusted for age , renal function and underlying disease. For EFS curves of included and excluded cohorts optimal exposure range was taken as reference for HR calculation.

Multivariate regression analysis

Table 3 shows the outcome of the multivariate logistic and stepwise regression analysis. Within underlying diseases, it was observed that patients with auto-immune disease and multiple myeloma were significantly higher associated to be out target attainment in comparison to patients with acute leukemia. Next, for age category it was seen that children were significantly higher associated to be in target range in comparison to adults. Lastly, moderate renal function was significantly higher associated to be out target attainment in comparison to patients with a good renal function. From stepwise regression variables age, renal function and underlying disease were included as determinants in the final model.

Table 3 Multivariable logistic regression and forward-backward stepwise analysis outcome target attainment

Multivariable logistic regression analysis outcome: TARGET attainment 15-25 mg*h/l	**Odd ratio (95% CI)**	**Both Stepwise regression analysis +/-**	**P-value**
Underlying disease:		+	
Acute Leukemia	**Reference**		
Auto-immune disease	0.041 (95% CI 0.001-0.566)		0.035*
Bone marrow failure	0.356 (95% CI 0.032-3.344)		0.366
Childhood malignancy	0.21 (95% CI 0.015-2.926)		0.232
Chronic leukemia	1.128 (95% CI 0.10-25.469)		0.924
High RISK MDS	1.14 (95% CI 0.29-5.08)		0.852
Immunodeficiency	0.157 (95% CI 0.016-1.104)		0.075
Lymphoma	0.611 (95% CI 0.179-2.045)		0.424
Metabolic/inborn error	0.282 (95% CI 0.03-1.65)		0.178
Multiple myeloma	0.15 (95% CI 0.02-0.43)		0.003*
Plasma cell leukemia	0.038 (95% CI 0.03-35.46)		0.981
Age category:		+	
Kids (<18 years)	6.686 (95% CI 1.18-53.90)		0.042*
Adults(>18 years)	**Reference**		
Renal category:		+	
Low (<50 ml/min)	0.29 (95% CI 0.03-1.82)		0.202
Moderate (50-90 ml/min)	0.215 (95% CI 0.08-0.53)		0.001*
Good (>90 ml/min)	**Reference**		

Discussion

Main findings

One of the main aims in this study was to evaluate the association of patient characteristics applied as inclusion criteria of TARGET RCT with fludarabine exposure. The purpose behind this was to see whether these criteria potentially declare the contrast in outcome between TARGET and retrospective study of Langenhorst. In addition, it was also analyzed to what extent these patient characteristics are predictive towards fludarabine exposure attainment. This research showed association of characteristics with fludarabine AUC and target attainment.

First, a statistical change was observed in AUC attainment in between both included and excluded cohorts. A significantly lower fraction of criteria included patients was suboptimal exposed (1.08%) in comparison to the criteria excluded cohort (10.53%). Despite a lower frequency of overexposure within the excluded cohort (40.00%), total AUC attainment in overexposure in between "included" and "excluded" cohorts remained insignificant.

For secondary outcome EFS, it was seen that patients from excluded cohort within optimal exposure range had a significantly lower risk on events such as NRM or relapse in comparison to overexposed patients. Notably for included patients, risk on event in between optimal- and overexposure was insignificant. It may be suggested that patients eligible to inclusion criteria of TARGET study are less amendable to be effective in optimal exposure in comparison to excluded cohort. In other words, it may implicate that probability on events of maintaining optimal exposure of fludarabine could be determined by certain patient characteristics. The number of patients from both cohorts was lower than original retrospective study of Langenhorst where primary outcome EFS was originally powered. Therefore, assumptions based on this outcome will remain explorative and require further research.

Lastly the multivariate analysis showed that patient characteristics such as renal function, age and underlying disease were predictive towards fludarabine target exposure attainment. For renal function it was found that patients with a moderate renal function were more associated to be out of target range in comparison to a good renal function. Furthermore, children were higher associated to be in target exposure in comparison to adults, whereas underlying diseases multiple myeloma and auto-immune disease were significantly higher associated to be out target. However, as auto-immune disease patients are children and consists of only 4 patients, it was questioned whether this was coincidence or not.

Comparison with existing literature

To explain association of patient characteristics towards fludarabine exposure, it was evaluated whether the outcome of the multivariable analysis fits with existing literature. Renal function as predictor for fludarabine exposure is according to expectation as literature showed fludarabine clearance is particularly done by kidneys [12,13]. Due direct correlation of clearance with AUC, this basically means that renal clearance may co-predict whether a patient could get suboptimal, optimal or above optimal exposed. Furthermore, an earlier PK-PD study proved correlation in fludarabine clearance and kidney function [9]. Due limited inclusion of renal impaired patients, it was however not possible to measure the accurate effect of this criterium on total exposure attainment. In case of underlying diseases, it remains unclear how this variable is predictive towards target attainment as multiple factors are involved. In case of age it must be mentioned that all children were diagnosed with other underlying diseases in comparison to adults. As this makes direct comparison complicated, it should be further explored into what extent children with same underlying diseases as adults would associate with fludarabine exposure.

Implications

As patient characteristics in this study appeared to be associated with fludarabine exposure (figure 2), it was implicated that TARGET inclusion criteria may create predisposition to become underexposed or overexposed. In other words, patient characteristics applied as TARGET criteria may determine fludarabine exposure in patients prior undergoing an allogenic-SCT. This may also suggest that differences in patient characteristics declare the difference in between the TARGET trial and retrospective study. This would mean that patient characteristics should be considered when fludarabine is targeted on a certain exposure in clinical practice. In addition, it may be also further hypothesized that optimal exposure range differs among patient characteristics. Still this part of the analysis is explorative as this study was not powered for secondary outcome EFS. Follow-up research including a higher number of patients with all characteristics should be considered to test the differences in effectivity.

Strengths and limitations

This study had however strengths and limitations. A strong point is that multiple samples were used as dependent variables in the PK-PD model for total fludarabine AUC estimation from an earlier study [8]. It is therefore highly reliable that the total distribution over cumulative fludarabine exposure represents the true total fludarabine exposure within the study population.

However, a limitation is the inclusion of a relatively low number of patients with a renal function below 40 ml/min. Although it is expected that patients with a low kidney function are in general not included at all, the effect of inclusion criterium of TARGET study was underestimated. To study the effect of impaired kidneys, it is therefore recommended to include a higher number of patients from each patient category for follow up research. Another limitation was that exposure groups in this study were defined based on events outcomes within same subset as was performed in an earlier PK study [Langenhorst et al]. As it exploratively seemed that patient characteristics may influence effectivity of fludarabine exposure on clinical outcomes, it should be taken into consideration whether a new study could be started to redefine new reference values for fludarabine exposure based on events in a different study population.

Conclusion

It is concluded that characteristics applied as inclusion criteria for TARGET study such as renal function, age and underlying disease are associated with fludarabine AUC attainment in patients undergoing allogenic SCT. Inclusion criteria of TARGET study may explain the difference of fludarabine exposure relation with clinical outcomes of the retrospective study from Langenhorst. However, based on outcome EFS further assumptions will be explorative. Therefore, an external validation is recommended in another patient population. Follow-up research including a higher number of patients from every subcategory of patients should be considered.

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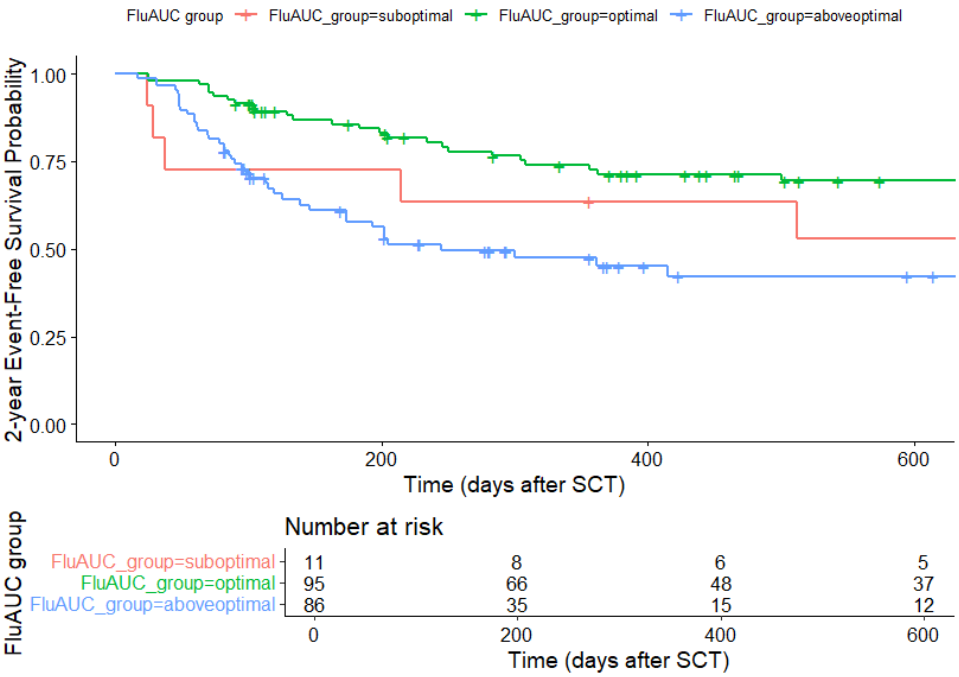
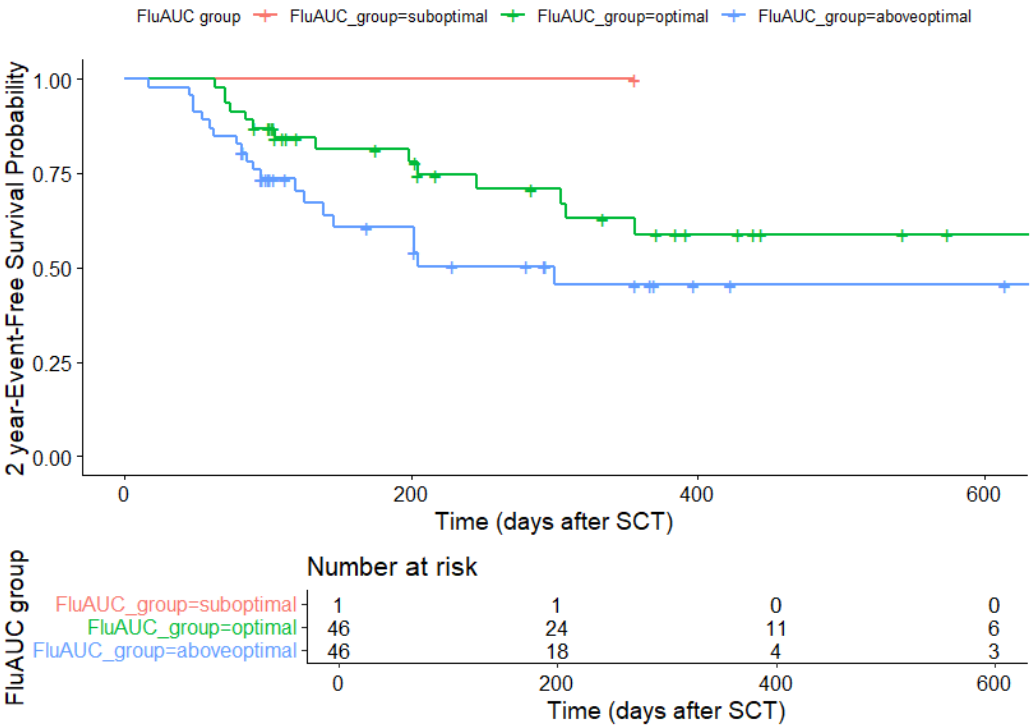
Appendix

Unadjusted Event-Free Survival Curves

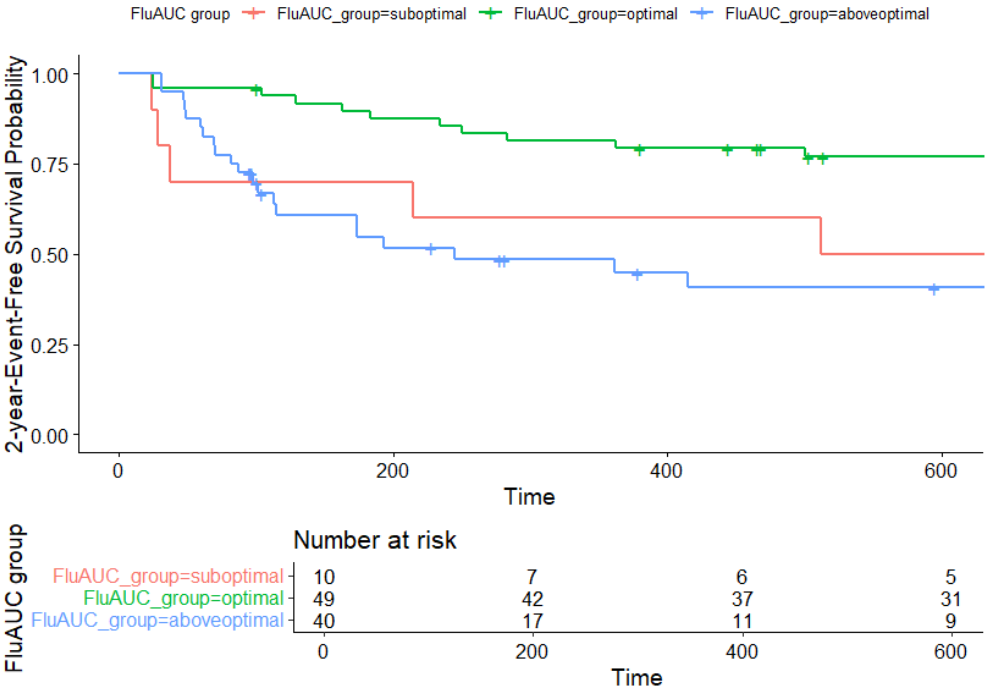
EFS curves from all population from retrospective cohort



Unadjusted EFS curves from Included cohort



EFS curves from Excluded cohort



Unadjusted Event-free-survival curve optimal exposure included vs excluded