RESULTS

Study title

Donor Regulatory T cells (T_{reg}) infusion (DTI) in patients with steroid-refractory chronic graft-versus-host disease (cGVHD)

EUDRACT N°

2012-000301-71

PI

Frédéric Baron, MD, PhD

Sponsor

CHU de Liège

Avenue de l'hôpital, 1

4000 Liège
Belgium

Table of contents

1. Patients

This trial was a pilot study of donor T_{reg} infusion (DTI) after initiation of the mTOR inhibitor rapamycin. Six patients received DTI (Table 1). Seven patients were included in the DTI group and one was not infused because the DTI product did not meet the release criteria (see below, patient PRE4).

The protocol was amended to a pilot study of donor T_{reg} infusion following discontinuation of the calcineurin inhibitor and initiation of the mTOR inhibitor rapamycin, including low-dose IL-2 (as reported by Koreth *et al.*[1]) given for 2 months starting just after DTI. Eight patients were enrolled in the amended protocol. However, several unexpected hurdles occurred that precluded T_{reg} infusion in 7 of these patients (donor refusal/unavailable (n=3), progression after rapamycin initiation before DTI (n=2) and DTI product not meeting the release criteria (n=2; CHUST-CHR_AMND5 and CHUST-CHR_AMND6). Ultimately, only one patient (CHUST-CHR_AMND3) received the T_{reg} product immediately followed by IL-2 administration ($1x10^6$ IU/day) for 2 months, under this amended protocol (Table 1).

Table 1. Patient characteristics

	PRE1	**PRE2**	**PRE3**	**PRE5**	**PRE6**	**PRE7**	**AMND3**
Patient age, years	44	35	52	36	68	64	57
Patient / donor gender	M/F	F/F	F/F	M/M	M/F	M/M	F/M
Donor type	Sib	Sib	MUD	MUD	MMUD*	Sib	Sib
Disease	AML	HD	MDS	HD	AML	CLL	HD
Conditioning regimen							
Myeloablative	Yes	Yes	Yes	Yes	No	No	No
Non-myeloablative	No	No	No	No	Yes	Yes	Yes
Total-body irradiation	Yes	Yes	No	Yes	Yes	Yes	Yes
ATG			Yes	No	No	No	No
Graft source	PBSC	PBSC	PBSC	PBSC	PBSC	PBSC	PBSC
HCT-CI score at transplantation	ND	ND	3	0	6	2	2
Days from cGVHD diagnosis to DTI	1894	910	263	182	496	631	1511

* 1 HLA-antigen mismatched in the GVHD direction (HLA-C); M, male; F, female; Sib, HLA-identical sibling; MUD, 10/10 HLA-allele matched donor; MMUD, 1 HLA-antigen/allele mismatched donor; PBSC, G-CSF-mobilized peripheral blood stem cells; AML, acute myeloid leukemia; HD, Hodgkin's disease; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukemia. DTI, donor T_{reg} infusion, ND, not done.

Their GVHD score at baseline is in the table 2 and the immunosuppressive drugs at infusion in the table 3.

Table 2. GVHD score at baseline (according to the NIH 2015 classification[2])

	baseline					
	skin	joints	mouth	eyes	Liver	Lung
CHUST_CHR_PRE1	3	1	3	2	1	0
CHUST_CHR_PRE2	3	3	1	2	0	0
CHUST_CHR_PRE3	1	3	0	2	0	0
CHUST_CHR_PRE5	0	2	0	0	0	0
CHUST_CHR_PRE6	2	0	0	0	0	2
CHUST_CHR_PRE7	0	0	0	3	0	0
CHUST_CHR_AMND3	3	0	0	1	0	0

Table 3. Concomitant immunosuppression at time of infusion

		Concomitant immunosuppression at time of infusion				
Patient	ECPhotopheresis	Steroids	Calcineurin inhibitors	Mycophenalate (MMF)	Sirolimus (rapamycin)	Imatinib
CHUST_CHR_PRE1	No	Yes	No	No	Yes	Yes
CHUST_CHR_PRE2	No	Yes	No	No	Yes	No
CHUST_CHR_PRE3	No	No	No	No	Yes	No
CHUST_CHR_PRE5	No	No	No	No	Yes	No
CHUST_CHR_PRE6	No	Yes	No	No	Yes	No
CHUST_CHR_PRE7	No	No	No	No	Yes	No
CHUST_CHR_AMND3	Yes	Yes	No	No	Yes	No

2. T_{reg} selection: phenotypic features

Pre-amendment

The initial leukapheresis product contained a median of 137 x 10^8 (range, 87-220 x 10^8) viable white blood cells (WBC). After the 2-step selection procedure, a median of 1.3 x 10^8 (range, 0.6-1.9 x 10^8) viable cells was recovered and median viability (determined by PI staining in flow cytometry) was 97.1 (range 65.5-99) %. Median % of $CD4^+CD25^+CD127^{dim/low}$ and $CD4^+CD25^+FoxP3^+$ cells in the final products assessed with the Miltenyi Biotec antibodies were 74 (range, 58-82), and 58 (range, 36-82), respectively, with consistently lower figures in the 5th patient who had a low viability. We attributed this low viability to the long delay between apheresis and start of the selection procedure (50h30 due to the fact that the apheresis product was collected in the US). Thus, release criteria were met for 6 of the 7 products while apheresis product from patient #5 failed to meet the criteria product because of low viability (65.5% instead of >80%) and low percentage of T_{reg} (35.7% instead of ≥ 55%) in the final product (and was thus not infused).

Post-amendment

Post-amendment, donor T cells were collected in 3 donors. Regarding the donor of patient AMND3, the initial leukapheresis product contained a median of 333 x 10^8 viable WBC. After the 2-step selection procedure, 1.3 x 10^8 viable cells was recovered and median viability

(determined by PI staining in flow cytometry) was 90%. Percentages of CD4+CD25+CD127$^{dim/low}$ and CD4+CD25+FoxP3+ cells were 84% and 57%, respectively. As mentioned above, unfortunately for two donors the release criteria of the T_{reg} product were not met: in the donor of patient AMND5, the initial leukapheresis product contained a median of 125 x 10^8 viable WBC. After the 2-step selection procedure, 2.2 x 10^8 viable cells was recovered and median viability (determined by PI staining in flow cytometry) was 93%. Percentages of CD4+CD25+CD127$^{dim/low}$ and CD4+CD25+FoxP3+ cells were 60% and 38% (below the release criteria of 55%), respectively and consequently the product was not infused; in the donor of patient AMND6, after the 2-step selection procedure, 1.5 x 10^8 viable cells was recovered and median viability (determined by PI staining in flow cytometry) was 96%. Percentage of CD4+CD25+FoxP3+ cells was 45% (below the release criteria of 55%), respectively and consequently the product was not infused either.

3. Safety

3.1. Infusion-related toxicity

Pre-amendment

There was no immediate toxicity associated with DTI. However, one of the 6 DTI recipients (patient PRE6) experienced a severe worsening of his skin GVHD one week after DTI (with a very inflammatory aspect). This is the only patient who received cells from a 1 HLA-antigen mismatched donor. The patient was taken out of the study and was treated with 1 pulse of cyclophosphamide (1g/m^2) and with everolimus (and discontinuation of sirolimus) with the aim of favouring T_{regs}. This was complicated by lung aspergillosis. Three months later the inflammatory aspect of the skin was resolved and no other signs of GVHD were present. However the general condition of the patient was dramatically impaired. Two weeks later the skin had taken again an inflammatory aspect (in a context of infra-therapeutic blood levels of everolimus) and the patient was admitted for poor general condition. Although the GVHD of the patient improved, the patient developed recurrent episodes of left lung atelectasia due to mucous plugs and eventually unfortunately succumbed from complications (liver bleeding) in ICU subsequent to a 4th episode of lung atelectasia.

Post-amendment

T_{reg} infusion and IL-2 were well tolerated in patient AMND3. The patient had stable disease while DTI + IL-2 administration resulted in dramatic T_{reg} increase in the blood during the 2 months of IL-2 administration.

3.2. Infections

Three patients experience infectious complications during the first year after donor T_{reg} infusion (Table 4). Two episodes were considered possibly and probably related in the "CHUST_CHR_PRE6" patient since it occurred in the context of GVHD exacerbation and increased immunosuppressive treatment.

Table 4. Infections occurring the first year after donor T_{reg} infusion

Individual	Months	AE	Related to T-reg
CHUST_CHR_PRE5	6-12	Upper respiratory tract infection	Not related
CHUST_CHR_PRE6	0-1	Lung infection	Possibly related
CHUST_CHR_PRE6	3	Lung infection	Probably related
CHUST_CHR_AMND3	0-3	Upper respiratory tract infection	Not related

3.3. Mortality during study

One patient ("CHUST_CHR_PRE6" treated before the protocol amendment) died during the first year after donor T_{reg} infusion. As mentioned above, this was attributed as probably related to the donor T_{reg} infusion.

The list of SAE occurring during the study period is listed in the table 5.

Table 5. SAEs reported during the study

Patient	SAE	Day after DTI	Relation with DTI	Other
CHUST_CHR_PR#				
PRE1	None			
PRE2	IV line for photopheresis	323	Not related	
PRE3	None			
PRE5	None			
PRE6	Worsening of skin GVHD	7	Probably related	Treatment with cyclophosphamide IV
	Lung infection	18	Possibly related	Treatment with meropenem

	Lung infection & skin GVHD worsening (everolimus below therapeutic range)	98	Probably related	Admission to ICU and antibiotics
	Atelectasia of the left lung <- mucus plug and bacterial pneumoniae	109	Probably related	Admission to ICU, bronchoscopy and antibiotics
	Atelectasia of the left lung <- mucus plug	114	Probably related	Admission to ICU, bronchoscopy and antibiotics
	Atelectasia of the left lung <- mucus plug	139	Probably related	Admission to ICU, bronchoscopy and antibiotics; cardiac arrest and death on day 144
PRE7	None			
CHUST_CHR_AMND3	None			

4. Efficacy

The evolution of cGVHD based on the NIH 2015 criteria are listed in the figure 1. Of the 7 infused patients, one patient achieved A CR, one a PR, two had a stable disease, one had a mixed response and two progressed.

Figure 1. Summary of clinical responses

	baseline						follow-up								
	skin	joints	mouth	eyes	Liver	Lung	skin	joints	mouth	eyes	Liver	Lung	Visit	Response	Medications
CHUST_CHR_PRE1	3	1	3	2	1	0	3	1	2	2	1	0	12 mo	Stable	No change
CHUST_CHR_PRE2	3	3	1	2	0	0	3	3	1	2	0	1	6 mo	PD	+MMF, +PCE, +Puva - Siro, - steroids
CHUST_CHR_PRE3	1	3	0	2	0	0	1	2	0	1	0	1	12 mo	MR	+ steroids
CHUST_CHR_PRE5	0	2	0	0	0	0	0	0	0	0	0	0	12 mo	CR	No change
CHUST_CHR_PRE6	2	0	0	0	0	2	3	0	0	0	0	2	3 mo	PD	- Siro, + Cy + Eve
CHUST_CHR_PRE7	0	0	0	3	0	0	0	0	0	2	0	0	12 mo	PR	Siro lowered
CHUST_CHR_AMND3	3	0	0	1	0	0	3	0	0	1	0	0	12 mo	Stable	No change

PD, progressive GVHD; MR, mixed response; CR, complete response; PR, partial response; PCE, photophoresis; Puva, psoralene + UVA; Siro, sirolimus (rapamune); Cy, cyclophosphamide; Eve, everolimus.

5. Conclusion

The conclusions are:

- Out of 10 manufactured products, seven met the release criteria,
- DTI was well tolerated in the 6 patients given DTI from 10/10 HLA-matched donors. In contrast, the patient receiving DTI from a HLA-mismatched unrelated donor had a worsening of his GVHD one week after DTI and eventually succumbed from infectious complications,
- The responses observed should be placed in the context of new therapies against chronic GVHD that became available after the trial such as ruxolitinib and belumosudil.

6. References

1. Koreth, J. *et al.* Interleukin-2 and regulatory T cells in graft-versus-host disease. *N Engl J Med* **365**, 2055–2066 (2011).
2. Jagasia, M. H. *et al.* National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant* **21**, 389-401.e1 (2015).