

T Cell Suicide Gene Therapy Prompts Thymic Renewal in Adults after Haploidentical Hematopoietic Stem Cell Transplantation in the Absence of post-Transplant Immunosuppression



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Abstract #1968

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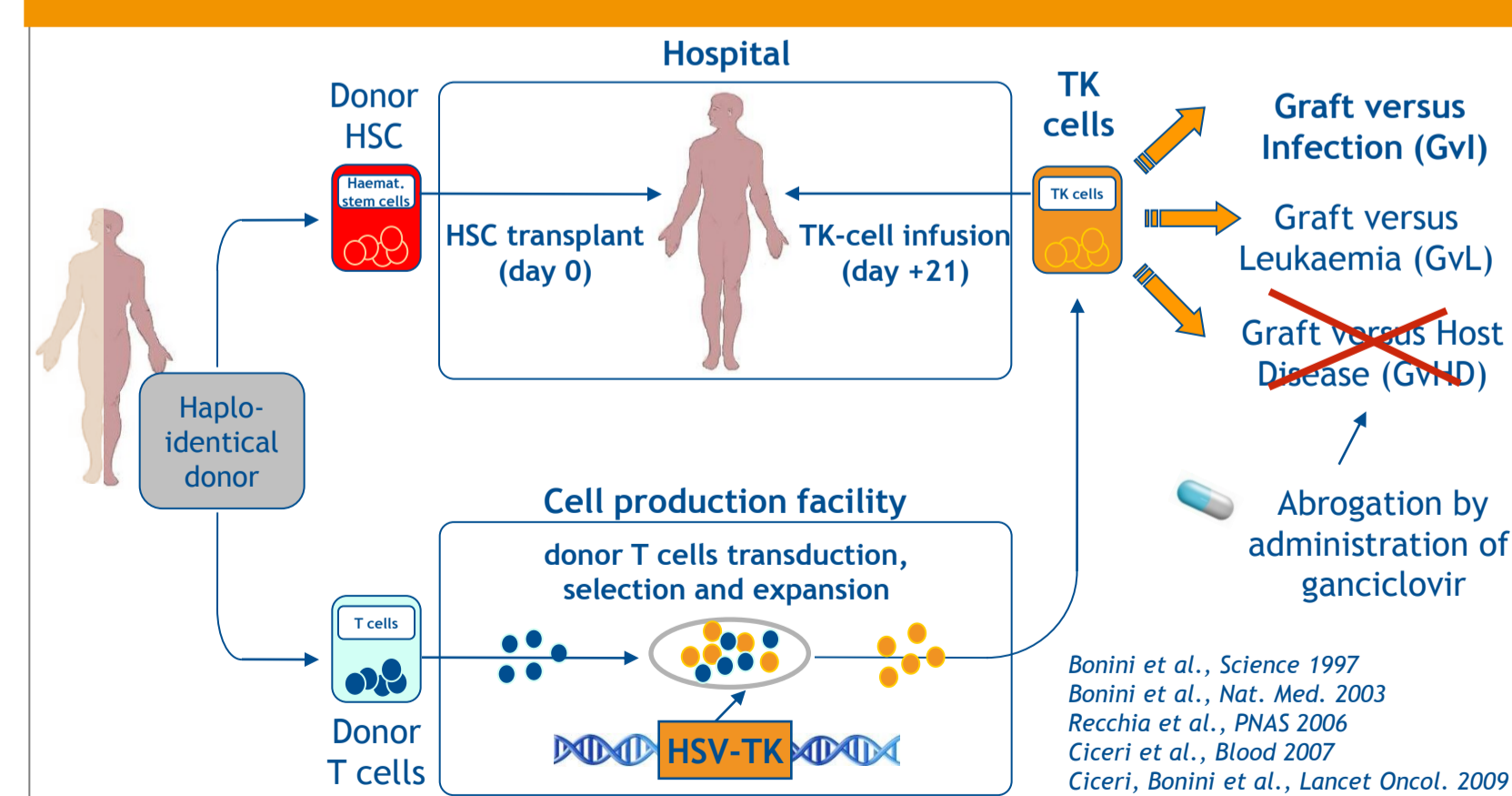
Abstract

BACKGROUND: The genetic modification of T cells with a suicide gene grants a mechanism of control of Graft-versus-Host Disease (GvHD), allowing safe infusion of donor lymphocytes after partially HLA-incompatible Hematopoietic Stem Cell Transplantation (HSCT). In the TK007 phase 1-2 clinical trial, which enrolled a total of 54 adults with hematologic malignancies, 22 of the 28 treated patients experienced a rapid and sustained immune recovery after T cell-depleted HSCT and serial infusions of purified donor T cells expressing the Herpes Simplex Virus Thymidine Kinase suicide gene (TK cells). In these patients, after a first wave of circulating TK cells, the majority of T cells supporting long-term immune reconstitution did not carry the suicide gene and displayed high numbers of Naïve lymphocytes, leading us to hypothesize a thymus-dependent development of T cells, occurring only upon TK cell engraftment.

METHODS: Thymic function was investigated in a total of 31 patients enrolled in the TK007 trial (median age 55 years), which were compared to a cohort of adult patients receiving non T cell-depleted haploidentical transplantation (n=31), and to healthy pediatric and adult subjects. T cell subsets and the proportion of CD31+ recent thymic emigrants (RTEs) amongst CD4+ Naïve T cells were measured by immunophenotypic analysis. Single joint T cell Receptor Excision Circles (sjTREC) were quantified by qPCR. The volume of the biologically active thymus was assessed by chest CT scans. Serum concentration of cytokines was assessed by a multiplex luminex-based assay. Pathogen-specific immunity was quantified by interferon- γ ELISpot.

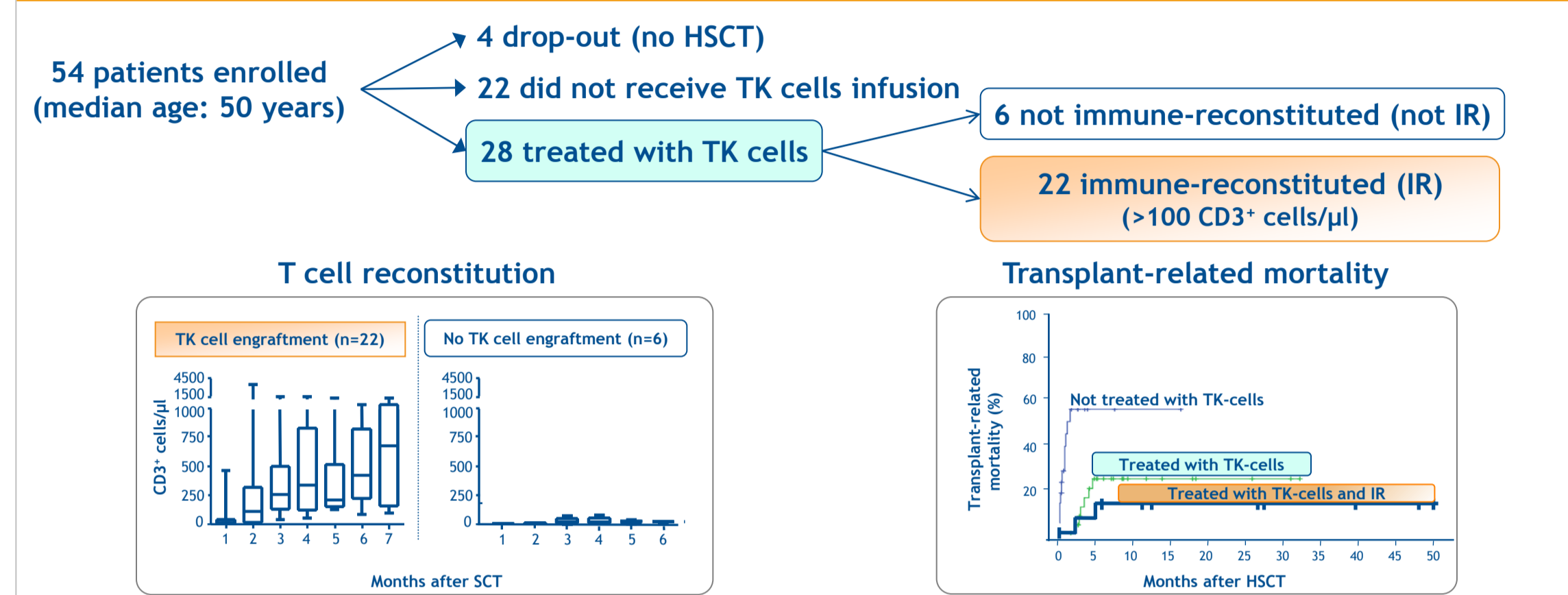
RESULTS: After the infusion of TK cells we documented a significant increase in peripheral blood sjTRECs as compared to the pre-HSCT determination (p = 0.02), suggesting an improved thymic output. Importantly, in line with that, only in TK007 patients almost the totality of CD4+ Naïve T cells circulating after transplantation were CD31+, thus bona fide recent thymic emigrants (89.54±9.55% at immune reconstitution, 81.84±15.9% at 6 months after HSCT, and 79.55±16.66% at 12 months after HSCT). Accordingly, a substantial expansion of the active thymic tissue was observed at chest tomography scans as compared to the pre-HSCT counterparts (p < 0.0001). A peculiar observation, possibly linked to the renewal of thymic activity and unique to the TK007 patients who achieved immune reconstitution, was the documentation of a peak in the serum level of interleukin-7, reproducibly occurring after each infusion of suicide gene-modified cells and anticipating the appearance of the newly generated T cells. Ultimately, the development of a wide repertoire of T cells in the patient thymus from donor precursors ensured a long-term protective immunity against pathogens, as exemplified by the preservation of a physiological and protective response against viruses both *ex vivo* and *in vivo*, even after the elimination of the infused TK cells in case of GvHD. **CONCLUSIONS:** Our data from TK007 patients show that the infusion of genetically modified donor T cells after transplantation can drive the recovery of thymic activity in adults, leading to long-term immune reconstitution. On the lead of the encouraging biological and clinical results of the phase I-II clinical trial (TK008 study) to assess the efficacy of TK cells in the context of haploidentical HSCT for leukemia started in 2010 at the San Raffaele Institute, and is currently expanding to multiple centers throughout Europe and US. Main endpoints of this randomized phase III trial are disease free survival and overall survival. The first TK008 patients randomized to receive suicide gene-modified cells showed recovery of thymic activity and concomitantly achieved a rapid and robust T cell immune reconstitution.

Figure 1. TK cell therapy in haploidentical-HSCT



- Donor T cells transduced with the Herpes Simplex Virus Thymidine Kinase gene (TK cells) are selected and expanded *in vitro*
- After 20-40 days from haploidentical HSCT, TK-cells are infused into the patient
- In case of GvHD patients are treated with ganciclovir

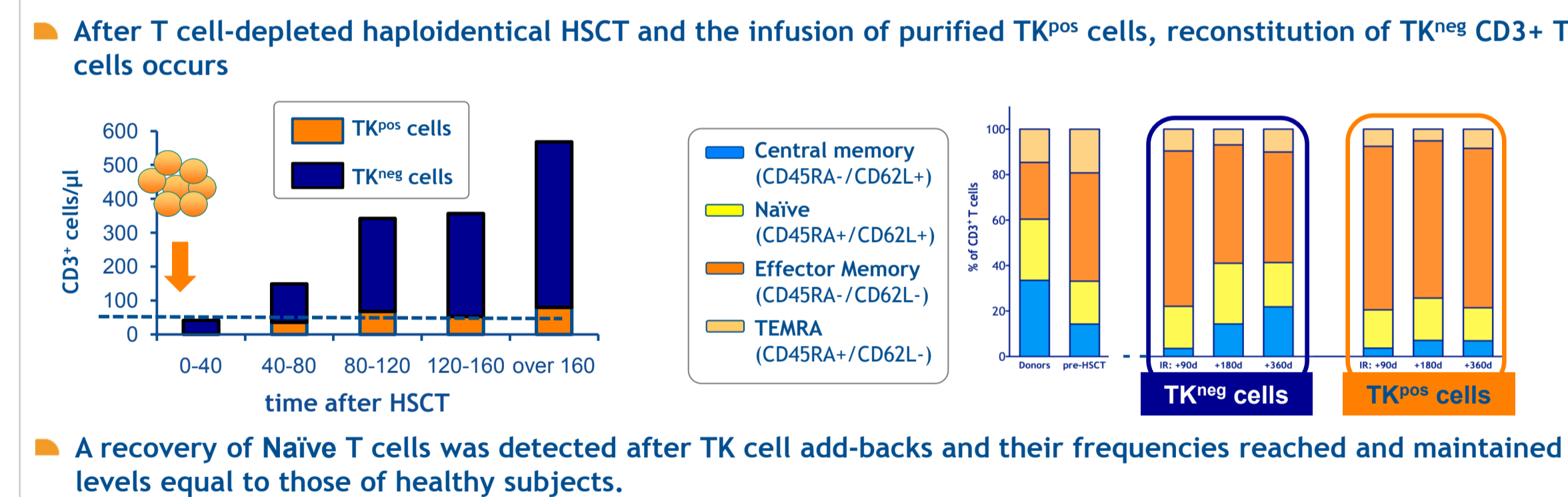
Figure 2. Phase II trial TK007: T cell reconstitution and transplant-related mortality



- Patients with TK-cell engraftment promptly and stably achieved CD3+ counts of 100 cells per μ l or more, whereas patients without TK-cell engraftment remained immuno-deficient

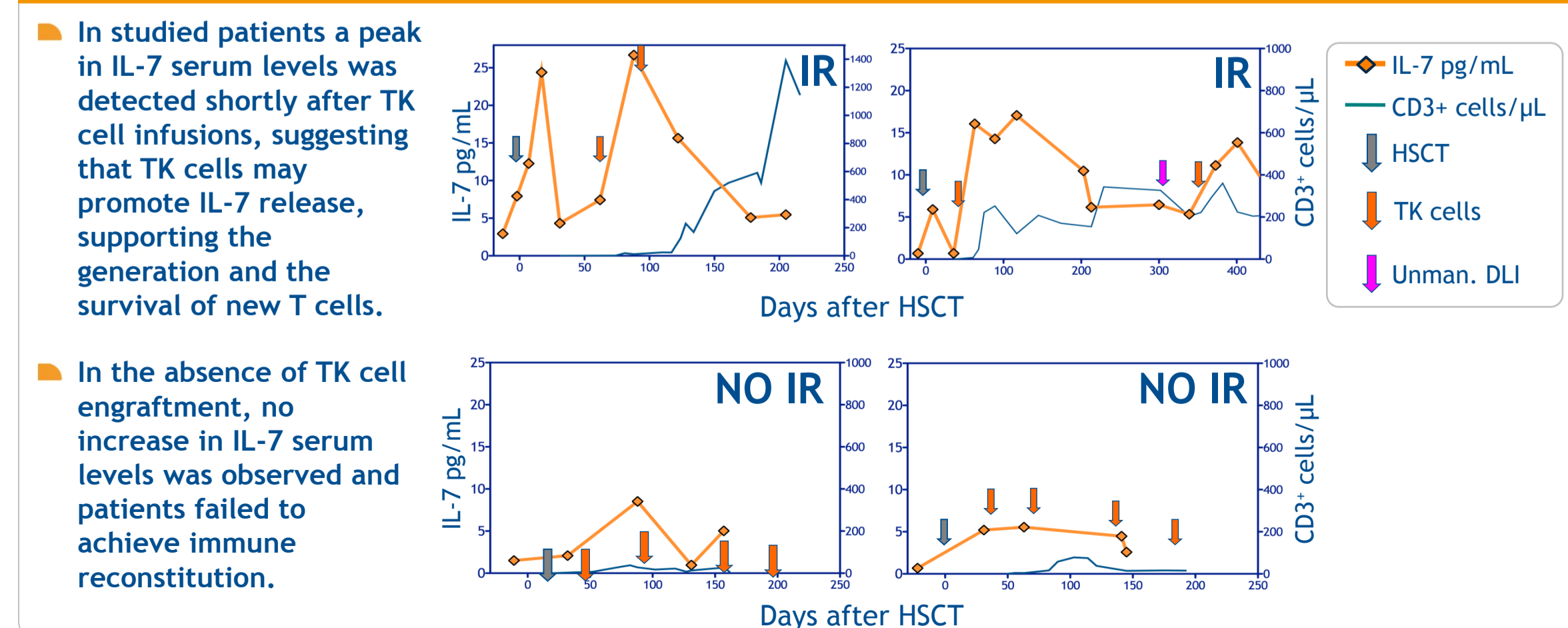
Ciceri, Bonini et al., Lancet Oncol. 2009

Figure 3. T cell reconstitution after HSCT and TK cell add-backs



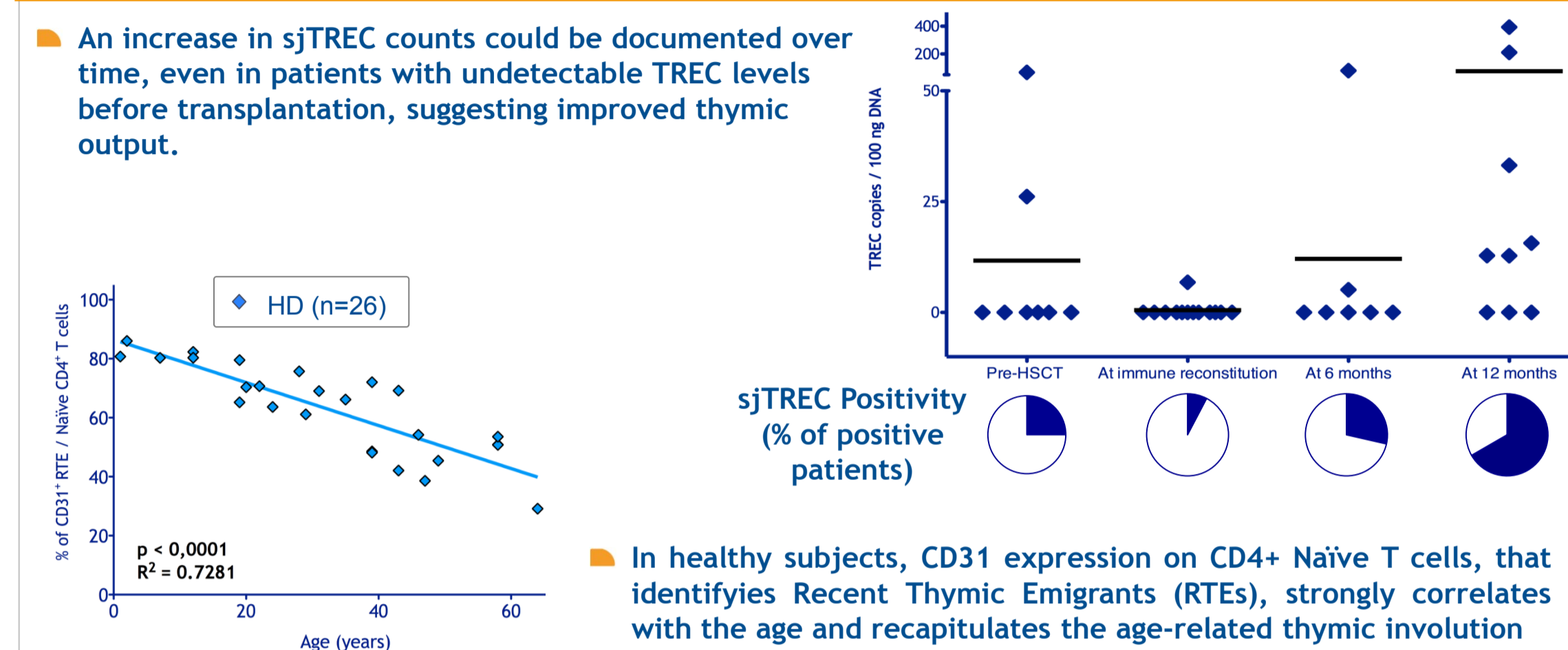
- After T cell-depleted haploidentical HSCT and the infusion of purified TK^{pos} cells, reconstitution of TK^{neg} CD3+ T cells occurs
- A recovery of Naïve T cells was detected after TK cell add-backs and their frequencies reached and maintained levels equal to those of healthy subjects.
- TK-positive cells represented the minority of circulating lymphocytes and presented a predominant Effector phenotype similar to the one of the infused cells, probably a result of the *ex vivo* manipulation.

Figure 4. Serum IL-7 concentration after HSCT and TK cell add-backs



- In studied patients a peak in IL-7 serum levels was detected shortly after TK cell infusions, suggesting that TK cells may promote IL-7 release, supporting the generation and the survival of new T cells.
- In the absence of TK cell engraftment, no increase in IL-7 serum levels was observed and patients failed to achieve immune reconstitution.

Figure 5. Thymic contribution to T cell recovery after infusion of TK cells



- An increase in sjTREC counts could be documented over time, even in patients with undetectable TREC levels before transplantation, suggesting improved thymic output.

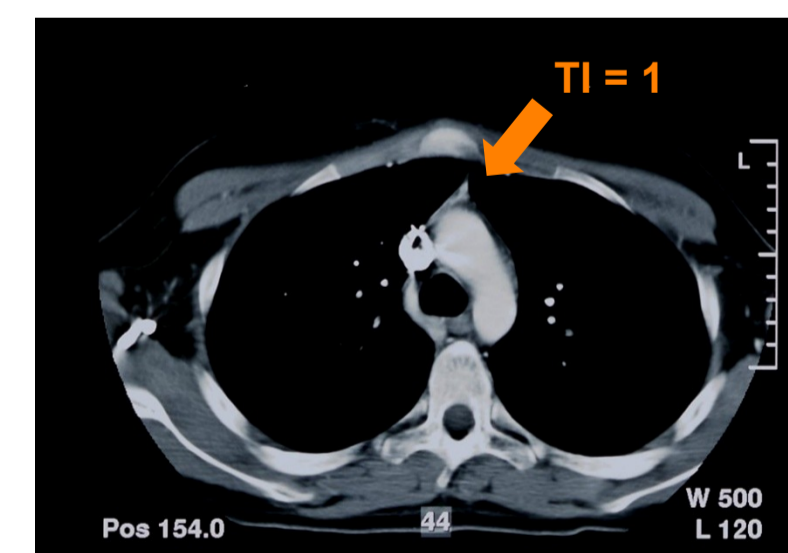
- In healthy subjects, CD31 expression on CD4+ Naïve T cells, that identifies Recent Thymic Emigrants (RTEs), strongly correlates with the age and recapitulates the age-related thymic involution

Recent Thymic Emigrants (RTEs) in TK007 patients

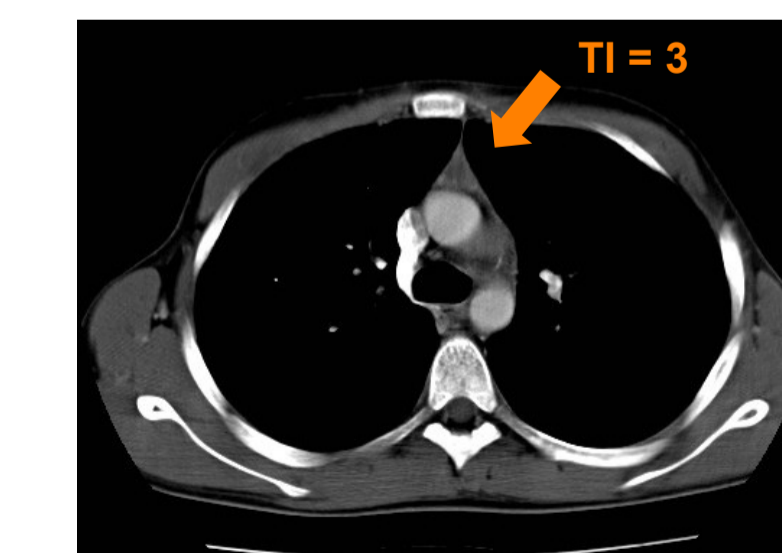
- Before HSCT, patients showed CD31 frequencies similar to that of age related healthy controls.
- At the time of the Immune Reconstitution, TK-neg donor-derived Naïve CD4+ T cells arising after TK cell infusions were mostly CD31 positive RTEs. RTE percentages remained high for months after transplantation in our patient cohort (median age: 50 years)
- In patients undergoing unselected T-cell replete HSCT, RTE frequencies 90 days after transplantation were significantly lower than the one detected after TK cell infusions, suggesting that this phenomenon is a direct consequence of the infusion of TK cells

Chest CT scans After TK-cell infusions

UPN #15 before TK cell infusion



UPN #15 after TK cell infusion



- Chest CT scans documented a significant increase in thymic index (TI) following TK cell infusion, in accordance with the hypothesis of renewal of biologically active thymic tissue

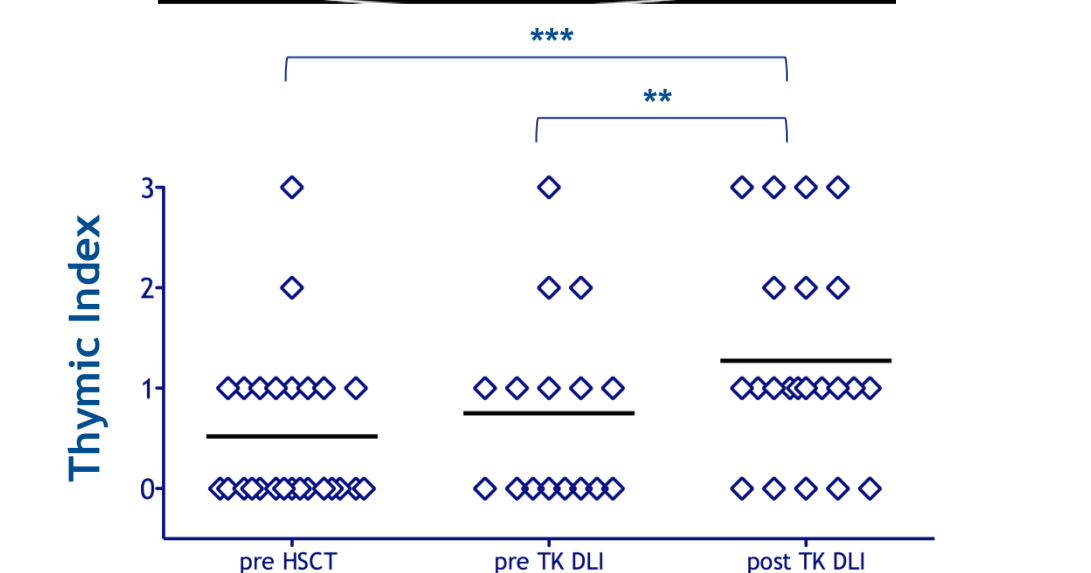
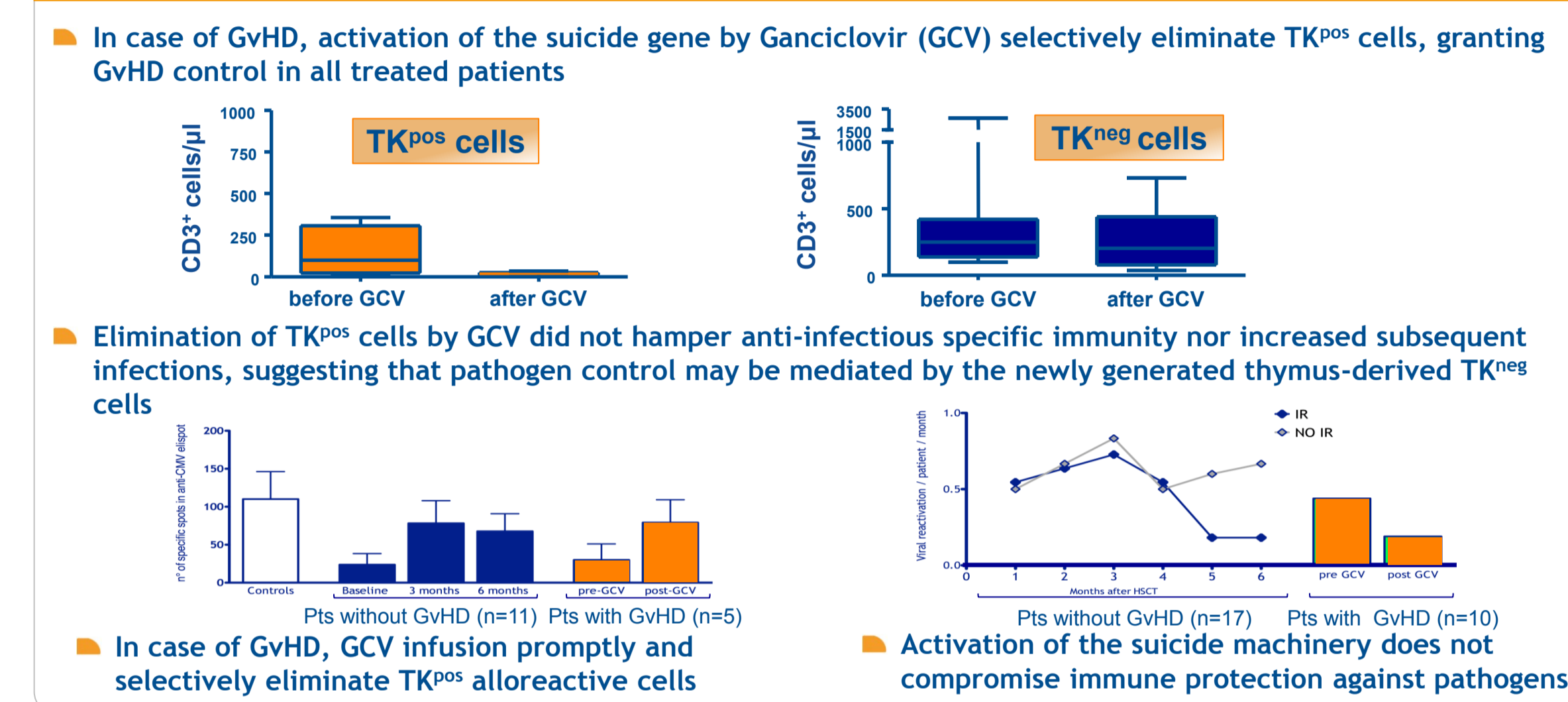


Figure 6. Protection against pathogens mediated by TK^{neg} cells



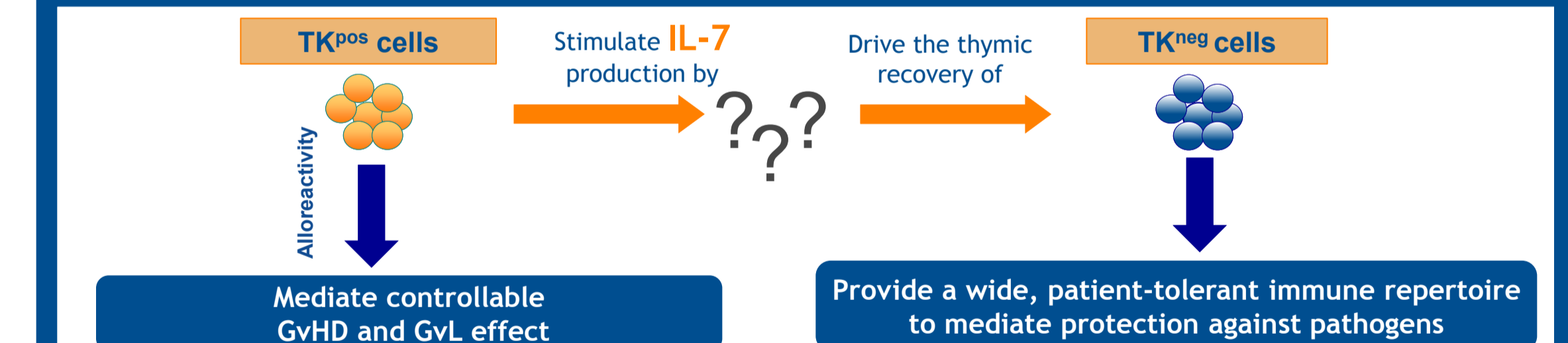
- In case of GvHD, activation of the suicide gene by Ganciclovir (GCV) selectively eliminate TK^{pos} cells, granting GvHD control in all treated patients

- Elimination of TK^{pos} cells by GCV did not hamper anti-infectious specific immunity nor increased subsequent infections, suggesting that pathogen control may be mediated by the newly generated thymus-derived TK^{neg} cells

- In case of GvHD, GCV infusion promptly and selectively eliminate TK^{pos} alloreactive cells
- Activation of the suicide machinery does not compromise immune protection against pathogens

CONCLUSIONS

- The infusion of genetically modified donor T cells prompts renewal of thymic activity, which contributes to recovery of a polyclonal T cell repertoire
- TK-cells act through an IL-7 dependent mechanism under investigation



- TK cells activity in the context of haplo-HSCT is currently being assessed in a Phase III clinical trial (TK008)

TK008 Phase III Clinical Trial

Key inclusion criteria:

- AML-ALL at high-risk in first CR
- AML-ALL in \geq second CR
- secondary AML in CR
- absence of HLA-matched family or unrelated donor

Stratification

- complete response (1st v $>$ 1st), performance status (0 v 1)
- country

Primary endpoint:

- disease-free survival

Secondary endpoints:

- non-relapse mortality, overall survival, immune-reconstitution, engraftment, aGvHD, cGvHD, relapse, disease-free survival, infectious, safety, quality of life, pharmacoeconomics

Contact persons for TK008 clinical trial:

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- Dr. Chiara Bonini, Experimental Hematology Unit, San Raffaele Scientific Institute, Milan, Italy; e-mail: bonini.chiara@hsr.it

CLINICAL CENTERS (15)

- EU (13):
 - Italy (2)
 - Germany (6)
 - France (1)
 - Greece (1)
 - Spain (1)
 - Belgium (2)
- Israel (1)
- US (1):
 - Illinois (1)

Study Design: N=127 (Haplo-HSCT + TK) vs N=43 (Haplo-HSCT)

- The first TK008 patients randomized to receive suicide gene-modified cells showed recovery of thymic activity and concomitantly achieved a rapid and robust T cell immune reconstitution. Immune recovery and absence of GvHD morbidity are so far confirmed, as compared to available options based on unmanipulated grafts from alternative donors such as cord blood