

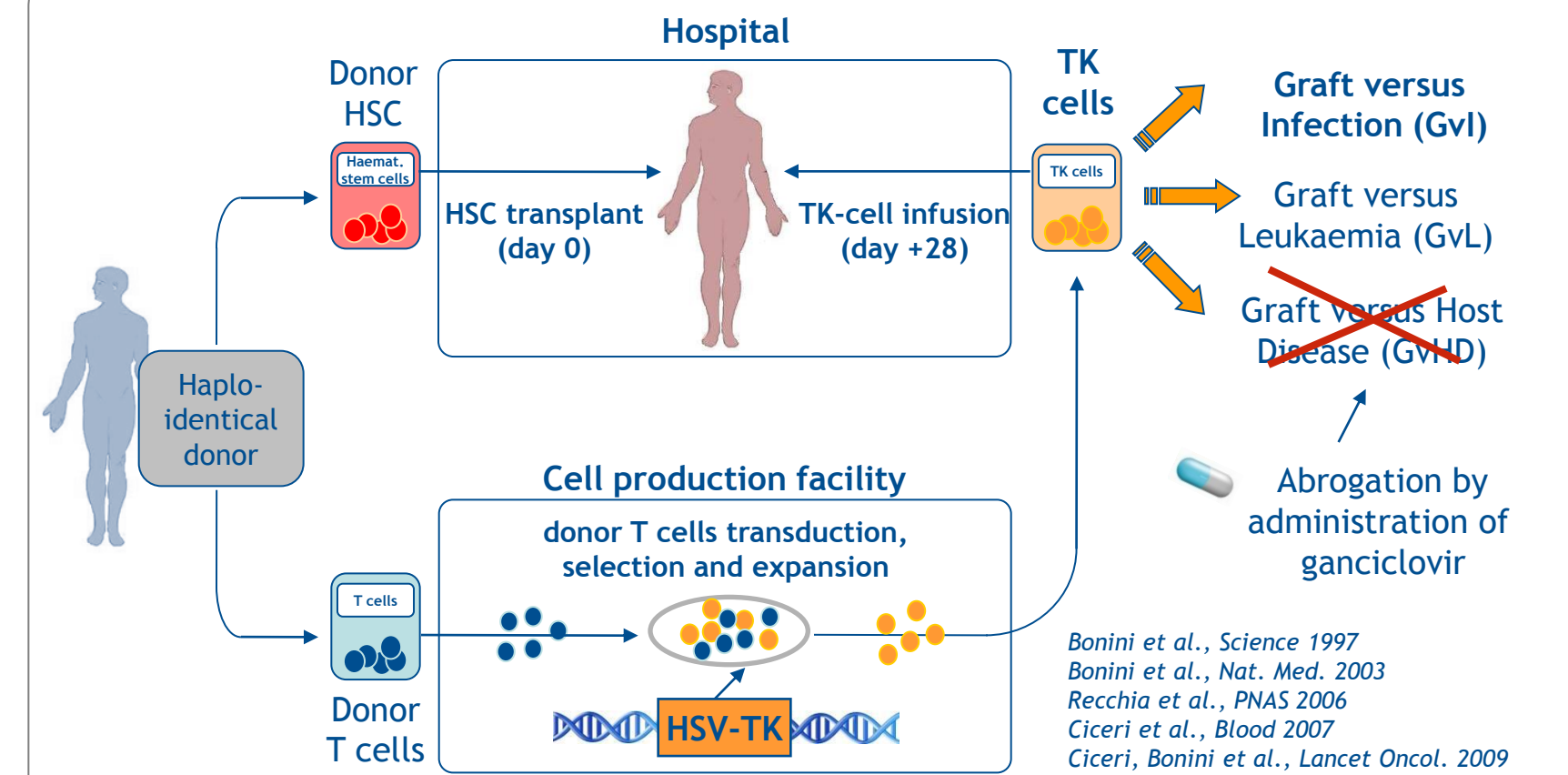
# Mechanism of thymic renewal after infusion of suicide gene-modified donor T cells after hematopoietic stem cell transplantation (HSCT) in adult patients

C. Bordignon<sup>1,6</sup>, L. Vago<sup>2</sup>, G. Oliveira<sup>2</sup>, M. Noviello<sup>2</sup>, C. Soldati<sup>4</sup>, D. Ghio<sup>4</sup>, I. Brigida<sup>5</sup>, A. Aiuti<sup>5</sup>, M.T. Lupo-Stanghellini<sup>2</sup>, J. Peccatori<sup>2</sup>, A. Lambiase<sup>6</sup>, A. Bondanza<sup>3</sup>, A. Del Maschio<sup>4</sup>, F. Ciceri<sup>2</sup>, C. Bonini<sup>3</sup>  
<sup>1</sup>Hematology, <sup>2</sup>Bone Marrow Transplantation Unit, <sup>3</sup>Experimental Hematology, <sup>4</sup>Department of Radiology, and <sup>5</sup>Division of Regenerative Medicine, Stem Cells and Gene Therapy, Istituto Scientifico San Raffaele, Milan, Italy; <sup>6</sup>MolMed, Milan, Italy

## Background

- Haematopoietic stem cell transplantation from partially HLA-matched family donors (haplo-HSCT) represents a promising therapy for high-risk leukaemia
- In haplo-HSCT, the infusion of TK cells - donor T cells genetically modified to express the Herpes Simplex Virus Thymidine kinase (HSV-TK) suicide gene - allows GvHD control, while rapidly providing effective and polyclonal T cell repertoire against pathogens and underlying tumours<sup>1</sup>
- Since newly reconstituting T cells were mostly TK<sup>neg</sup>, we hypothesized that TK cells, which are necessary to achieve this effect, might act by prompting T cell development from graft progenitors through a thymus-dependent pathway

Figure 1. TK cell therapy in haploidentical-HSCT

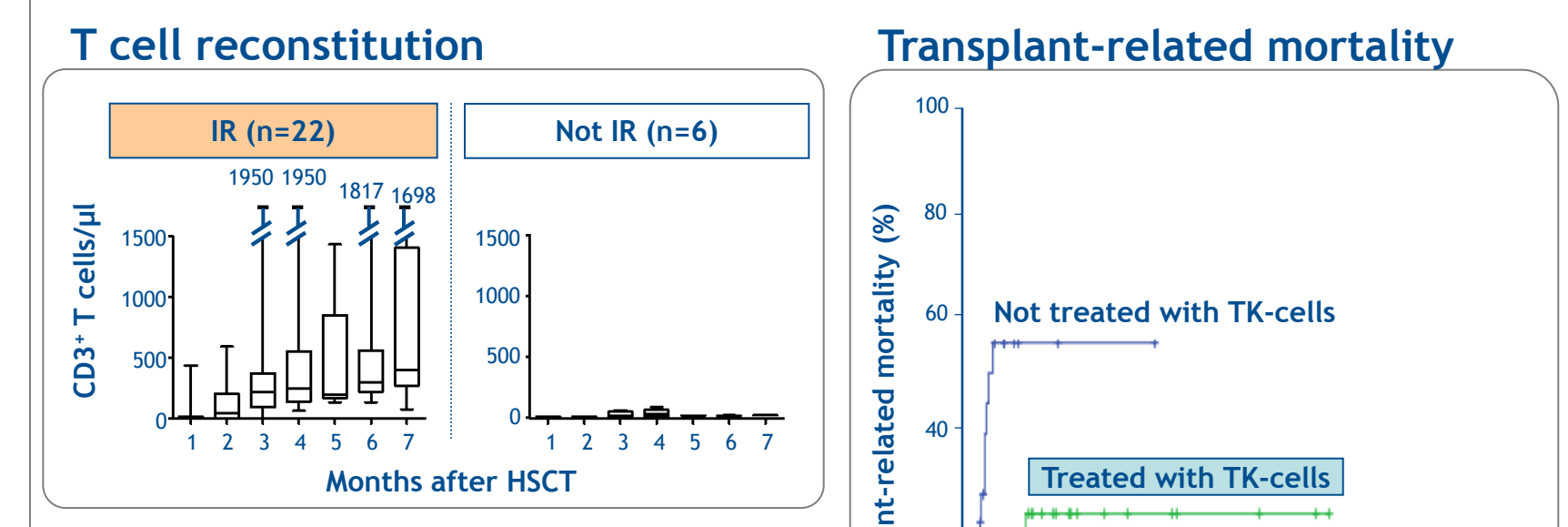
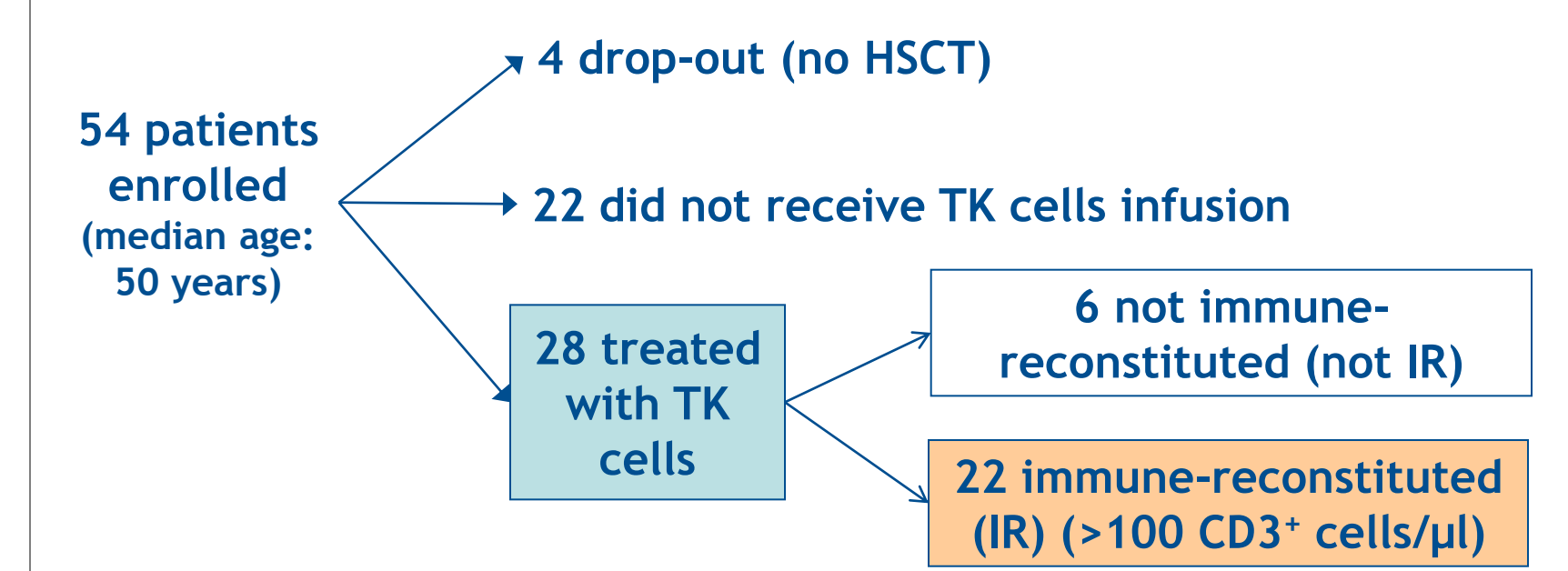


- Donor T cells transduced with the Herpes Simplex Virus Thymidine Kinase gene (TK cells) are selected and expanded *in vitro*
- After 20-40 days from haploidentical HSCT, TK-cells are infused into the patient
- In case of GvHD patients are treated with ganciclovir

## Methods

- In a Phase II trial (TK007) of haplo-HSCT for high-risk leukaemia, 28 patients received TK cells (TK<sup>+</sup> donor T cells)
- In a selected subset of patients, thymic function was assessed by quantitation of CD31+ in CD4+ naïve T cells, and was correlated with thymic volume, assessed by CT scans
- IL-7 serum levels were quantified through luminex assay

Figure 2. Phase II trial TK007: T cell reconstitution and transplant-related mortality<sup>1</sup>



- Patients with TK-cell engraftment promptly achieved CD3+ counts of 100 cells per μl or more, at median time of 75 days from transplant (range 34-127) and 23 days from TK-cell infusion (range 13-42)
- Patients without TK-cell engraftment remained immunodeficient
- TK cells protect from transplant-related mortality

## Results

- Post-transplant recovery of TK-negative naïve T cells occurred, reaching values comparable to controls in approximately one year
- After infusion of TK cells, almost all circulating CD4+ naïve T cells were CD31+ Recent Thymic Emigrants, suggesting a direct role of the infused TK cells in promoting thymopoiesis. Accordingly, CT scans documented an increase of thymic volume
- IL-7 serum levels markedly rose after TK cell infusions, suggesting an IL-7 dependent mechanism

Figure 3. T cell Immune-Reconstitution after HSCT and infusion of TK cells

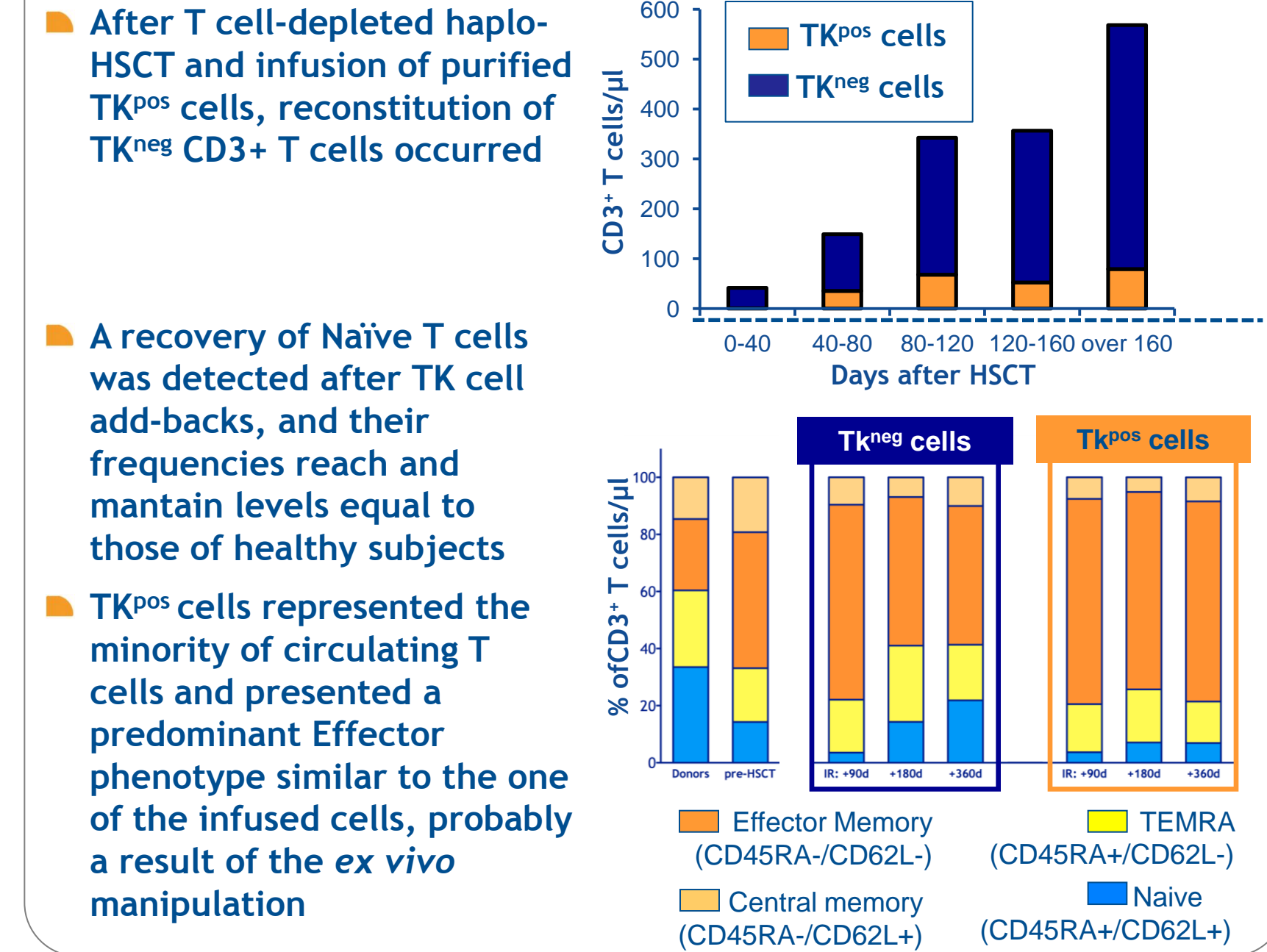
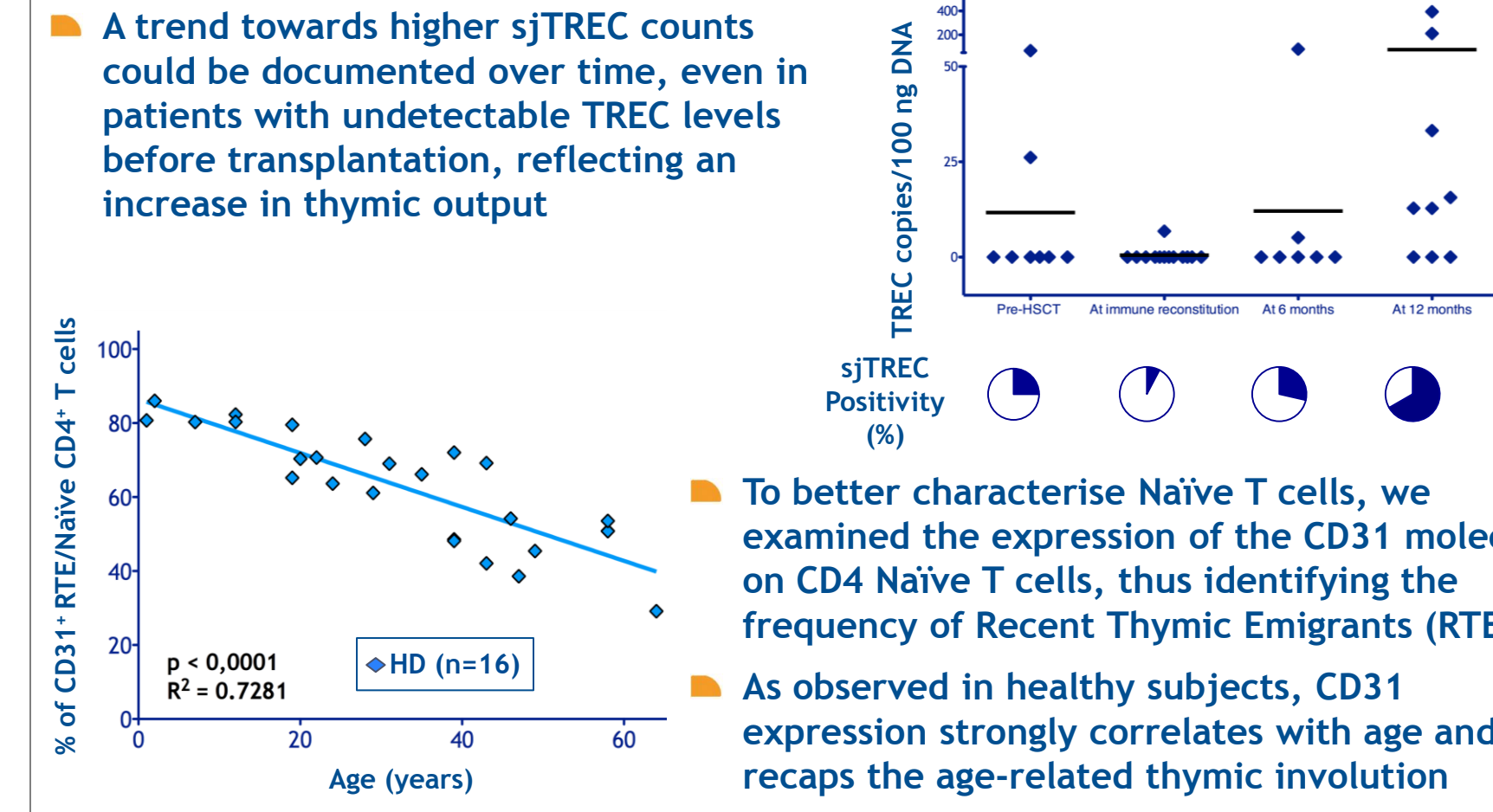


Figure 4. Protection against pathogens mediated by Tkneg cells

- In case of GvHD, activation of the suicide gene by Ganciclovir (GCV) selectively eliminate Tk<sup>pos</sup> cells, granting GvHD control in all treated patients
- Elimination of Tk<sup>pos</sup> 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<sup>neg</sup> cells
- GCV infusion promptly and selectively eliminate Tk<sup>pos</sup> alloreactive cells
- Activation of the suicide machinery does not compromise immune protection against pathogens

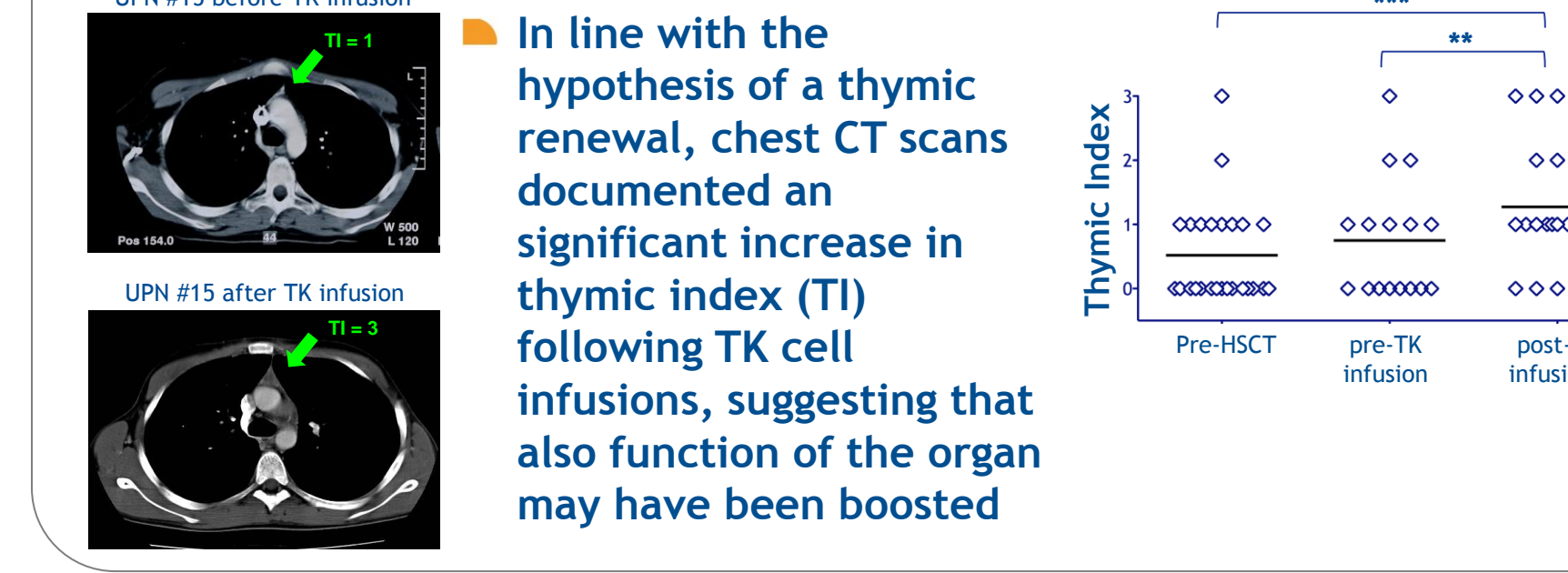
Figure 5. Thymic contribution to T cell recovery after infusion of TK cells



## Recent Thymic Emigrants (RTEs) in TK007 patients

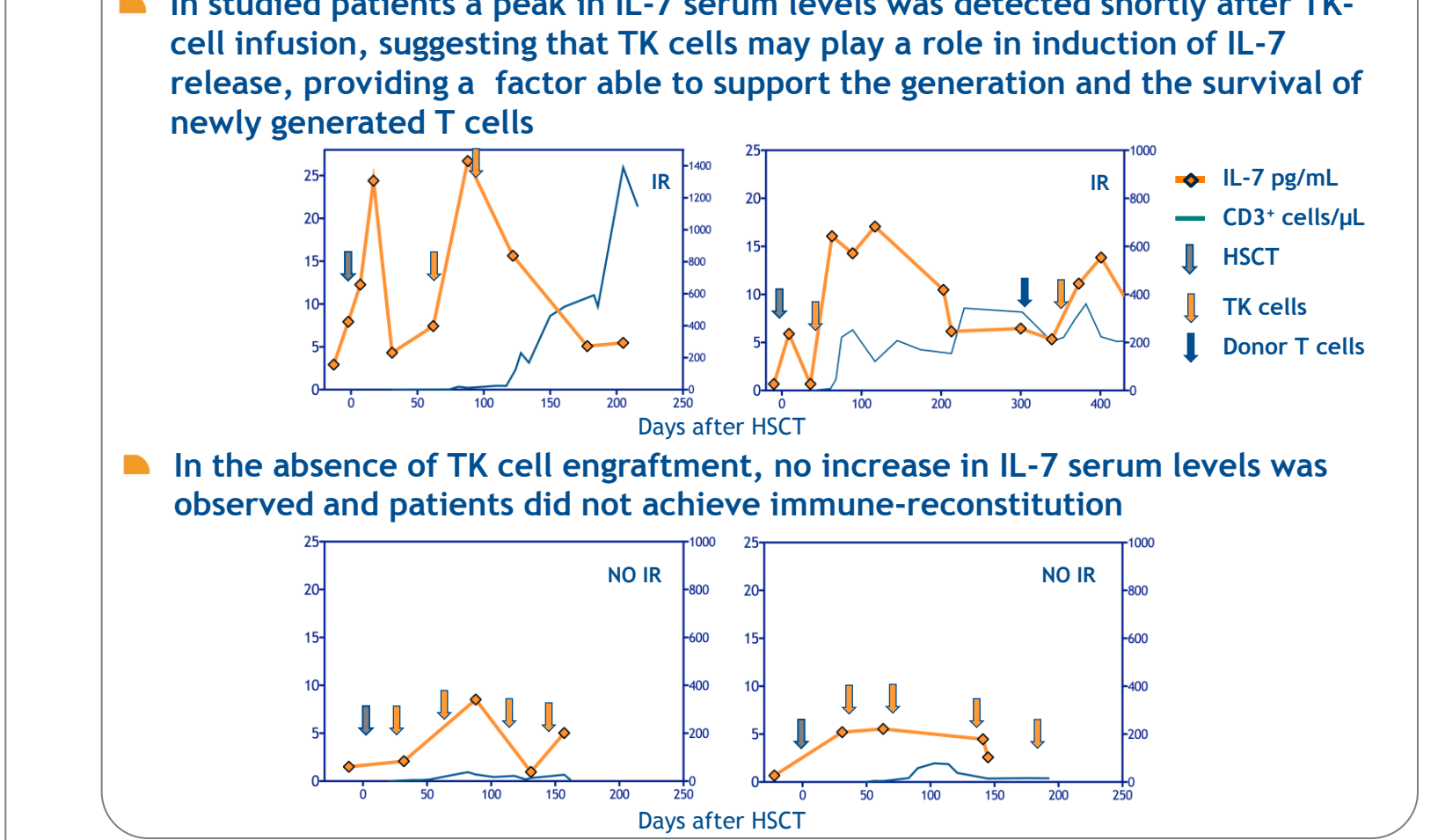
- Before HSCT, patients showed CD31 frequencies similar to that of age related healthy controls
- At the time of IR, TK<sup>neg</sup> donor-derived Naïve CD4+ T cells arising after TK cell infusions were mostly CD31+ RTEs. Their % remained high for months after HSCT, quite unexpectedly in patients with a median age of 50 years
- This phenomenon was a direct consequence of TK cells infusion, as supported by the study of a cohort of patients undergoing an unselected T-cell replete HSCT: the infusion of mature T cells within the donor graft is not able by itself to enhance RTE generation in the thymus, as demonstrated by the fact that RTE frequencies 90 days after HSCT were significantly lower than the one detected after TK cell infusion

## Chest CT scans after TK cell infusion



- In line with the hypothesis of a thymic renewal, chest CT scans documented a significant increase in thymic index (TI) following TK cell infusions, suggesting that also function of the organ may have been boosted

Figure 6. Serum IL-7 concentration after TK cells add-backs



## Conclusions

- The infusion of genetically modified donor T cells (TK 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)

## References

- Ciceri F., Bonini C. et al., *Lancet Oncol.* 2009 10(5): 489-500
- Kohler S. and Thiel A., *Blood* 2009 113(4): 769-74
- Vago L. et al., *N Engl J Med* 2009 361(5):478-488