GIORNATE EMATOLOGICHE VICENTINE

X edizione

12-13 Ottobre 2023
Palazzo Bonin Longare - Vicenza

Cellule autologhe redirette: stato dell'arte

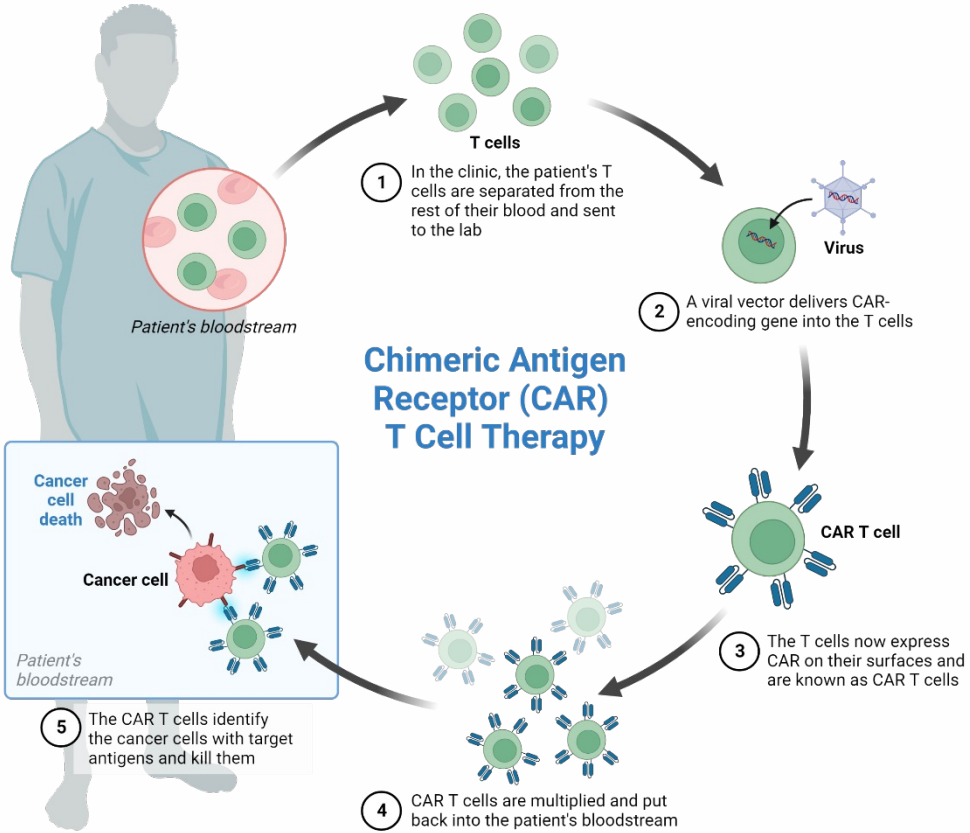
Antonio Rosato

Department of Surgery, Oncology and Gastroenterology, Immunology and Oncology Section, University of Padova and
Immunology and Molecular Oncology Unit, Veneto Institute of Oncology IOV – IRCCS, Padova, Italy

Disclosures of Name Surname

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
None to declare							

CAR T Cell Therapy



(axicabtagene ciloleucel) Suspension for IV infusion

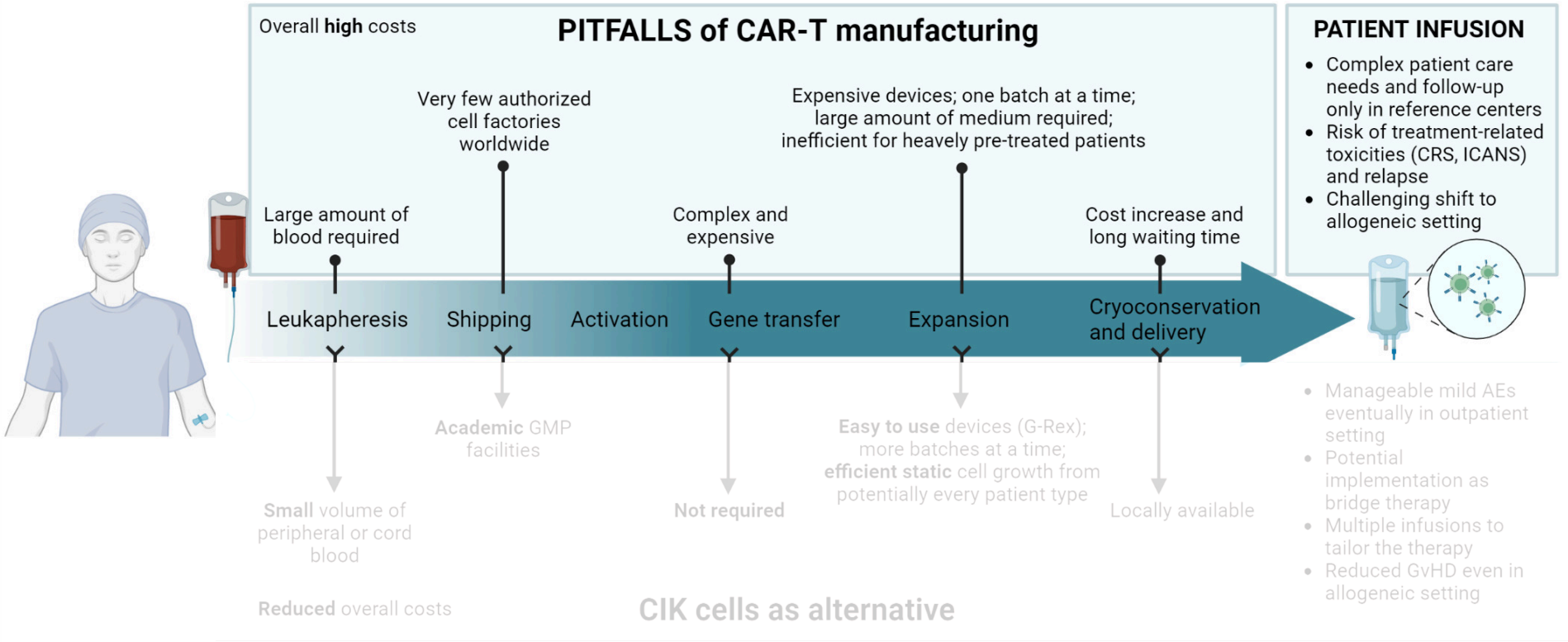
(tisagenlecleucel) Suspension for IV infusion

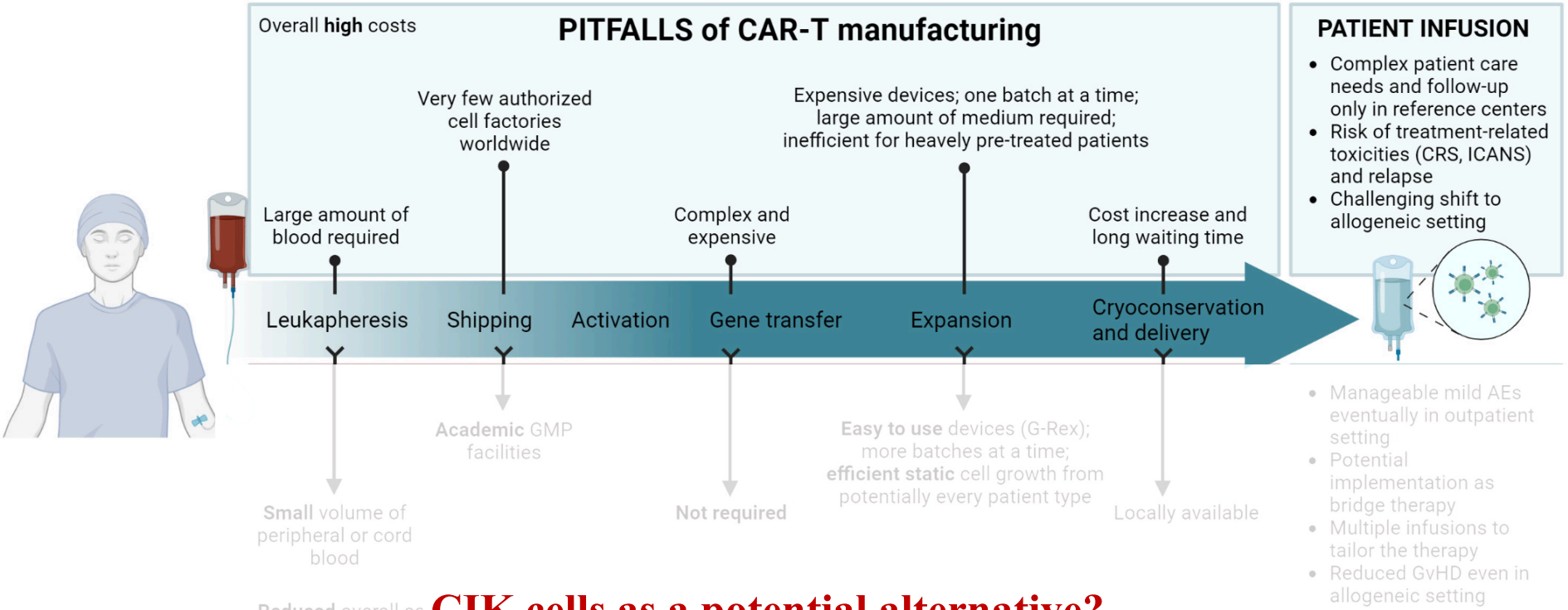
(brexucabtagene autoleucel) Suspension for IV infusion

(idecabtagene vicleucel) Suspension for IV infusion

(lisocabtagene maraleucel) Suspension for IV infusion

(ciltacabtagene autoleucel) Suspension for IV infusion

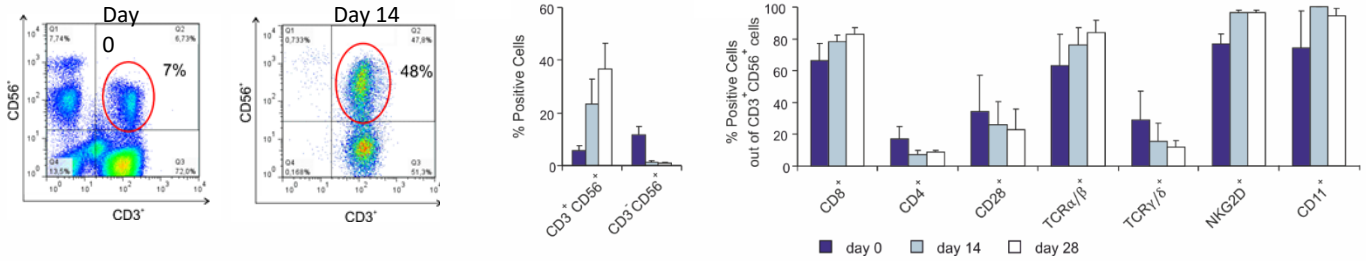

Overall high costs
PITFALLS of CAR-T manufacturing
PATIENT INFUSION
Very few authorized cell factories worldwide
Expensive devices; one batch at a time; large amount of medium required; inefficient for heavely pre-treated patients
Large amount of blood required
Complex and expensive
Cost increase and long waiting time
Complex patient care needs and follow-up only in reference centers
Risk of treatment-related toxicities (CRS, ICANS) and relapse
Challenging shift to allogeneic setting
Leukapheresis
Shipping
Activation
Gene transfer
Expansion
Cryoconservation and delivery
Academic GMP facilities
Easy to use devices (G-Rex); more batches at a time; efficient static cell growth from potentially every patient type
Manageable mild AEs eventually in outpatient setting
Potential implementation as bridge therapy
Multiple infusions to tailor the therapy
Reduced GvHD even in allogeneic setting
Small volume of peripheral or cord blood
Not required
Locally available
Reduced overall costs
CIK cells as alternative



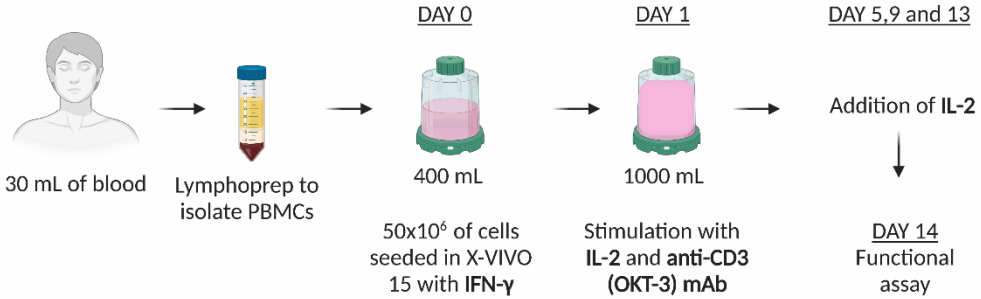
CIK cells as a potential alternative?

Background: CIK cells

- **Cytokine-Induced Killer (CIK) cells** are a heterogeneous population of ***ex-vivo*** **CD3⁺ CD56⁺ effector cells** with T and NK cell phenotypic and functional properties



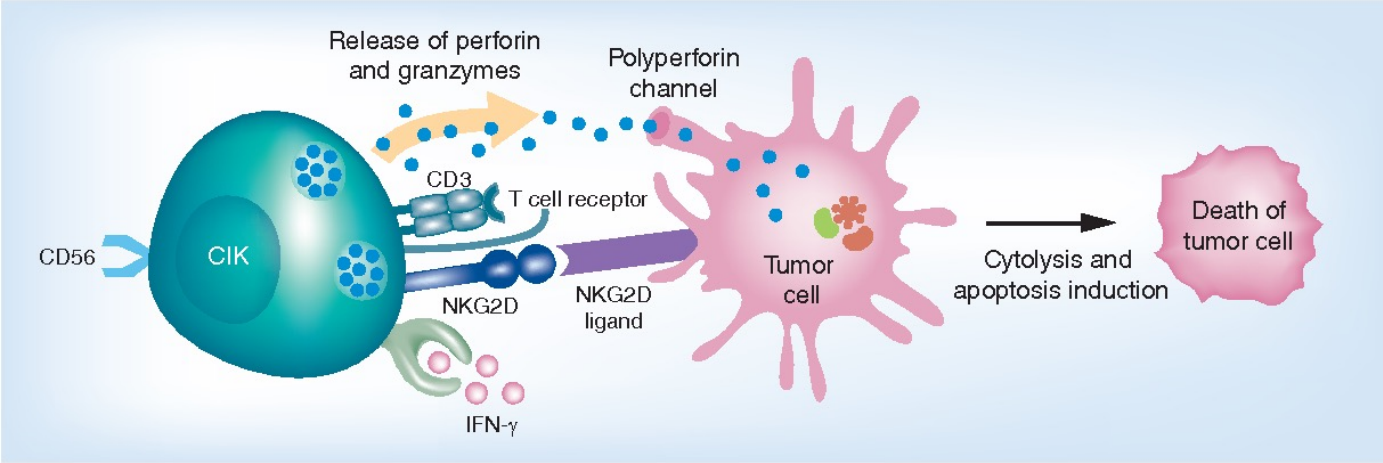
- Mature CIK cells are obtained in 14 days from PBMCs or cord blood by the addition of IFNγ, anti-CD3 mAb and IL-2



Background: CIK cells

ANTITUMOR ACTIVITY:

- ✓ NKG2D-mediated killing, no priming, MHC-independent cytotoxicity
- ✓ Feasibility of large-scale expansion
- ✓ Safety: reduced alloreactivity → reduced GVHD and low side effects.



Report 2020:

- 106 clinical trials enrolling 10,225 patients
- 4,889(47,8%) were treated with CIK alone or in combination therapies

- 30 kinds of cancers

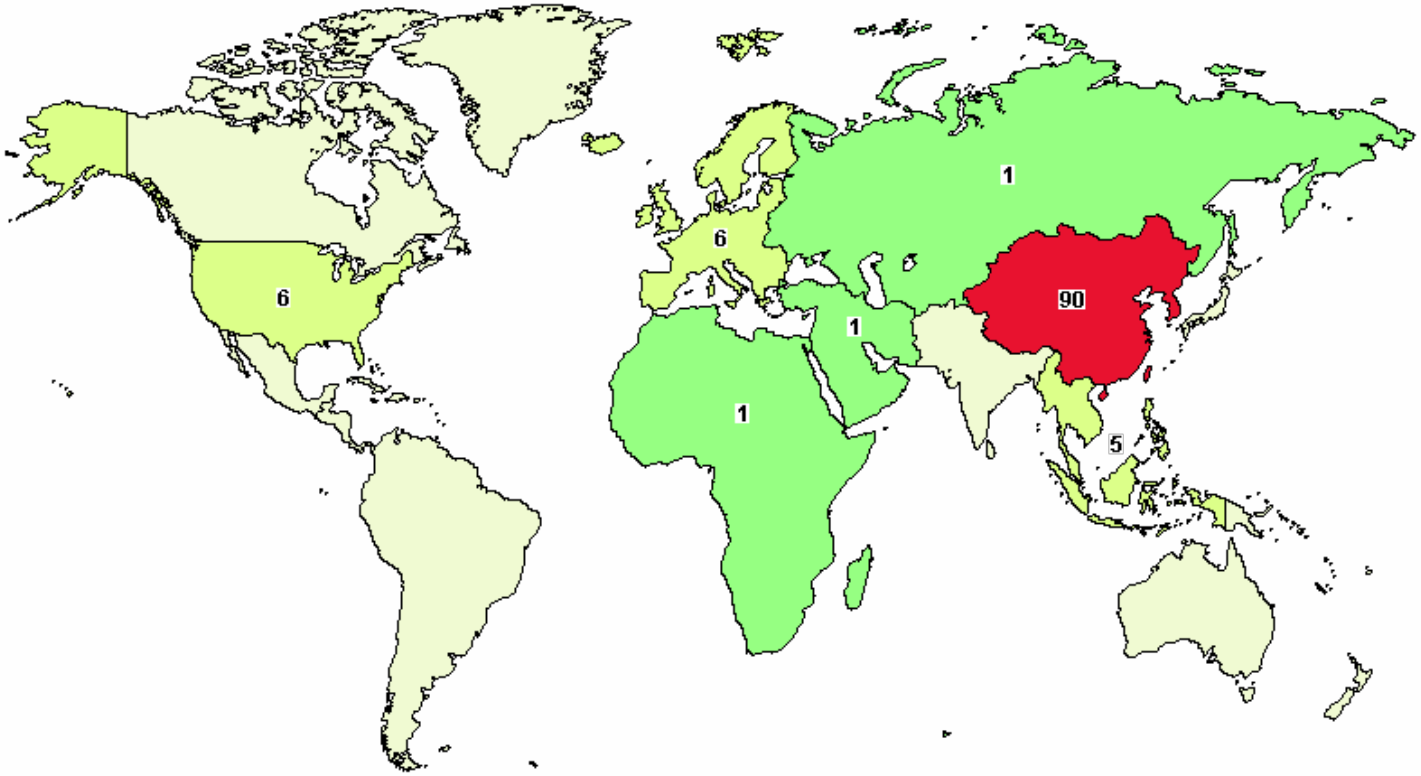
- Significant improvement of median progression free survival (**mPFS**) and median overall survival (**mOS**)

- 10 studies→ increased 1-year survival rate
- 9 studies→ increased 5-years survival rate

TABLE 1 Clinical trials based on CIK cell immunotherapy.

Reference	Tumor type	CIK-treated patients (n)	Treatment schedule	Efficacy	Safety
Laport et al. (81)	relapsed allo-HSCT	18	dose-escalating, from 1x10^7 CD3$^+$ cells/kg to 1x10^8 cells/kg	mOS: 28 months CR: 27.7%	aGVHD grade 1/2: 11% cGVHD: 5.5%
Narayan et al. (97) NCT01392989	Myeloid Neoplasms	44	one CIK cell infusion (12.4x10^8/kg) after conditioning	2-year OS: 52.6%	aGVHD: 16.3%
Merker et al. (98)	relapsed allo-HSCT	36	CIK cells (16x10^6/kg), median of 2 and maximum of 9 cycles	CR: 53%	aGVHD: 25%
Introna et al. (54)	relapsed allo-HSCT	73	sequential infusion of DLI (1x10^6/kg) followed by dose-escalating CIK cells (1 to 5x10^6/kg), for 3 cycles	CR: 26%, PR: 4%, stable disease: 11%. 1- and 3-year PFS: 31% and 29%. 1- and 3-year OS: 51% and 40%.	aGVHD: 16%
Wang et al. (56)	NSCLC	133 (auto) 170 (allo)	autologous or haploidentical, CIK cells 5x10^9 cells/cycle, 4 cycles	mOS: auto 11 months, allo 8 months	mild AEs, no differences allo vs auto (P>0.05)
Lee et al. (99) NCT00699816	HCC	114	autologous CIK cells, 6.4x10^9 cells/cycle,16 cycles in total	mDFS: 44 months	AEs grade 1 or 2: 47%
Chen et al. (100)	HCC	102	1.0 to 1.5x10^{10} CIK cells per cycle, at least 4 cycles, transfused after tumor resection	1-, 3-, and 5-year DFS: 85.3%, 68.2%, and 60.4%. 1-, 3-, and 5-year OS: 99.0%, 93.0%, and 84.3%.	mild and self-limiting AEs
Li et al. (101)	NPC	112	GC followed by at least 4 cycles of CIK cells	mPFS: 21 months mOS: 32 months	no acute or chronic infectious cases

CIK cell clinical trials worlwide

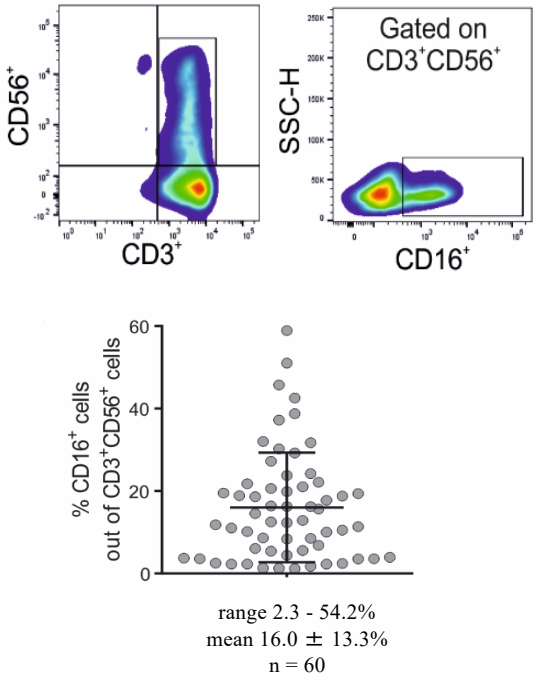


CIK cell clinical trials in Europe

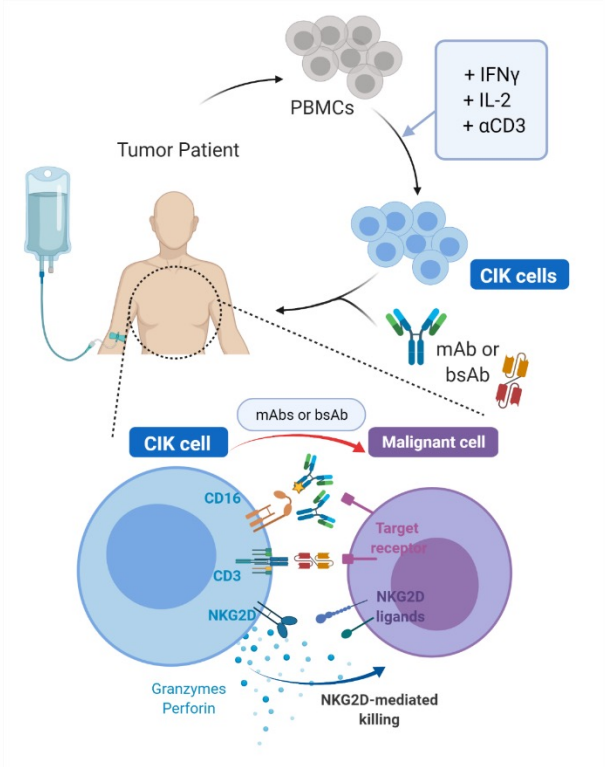
Row	Saved	Status	Study Title	Conditions	Interventions	Locations
1	☐	Recruiting	Infusion of Donor Derived Cytokine Induced Killer (**CIK**) Cells in Hematological Patients Relapsed After Haploidentical Stem Cell Transplant	• Relapsed Hematologic Malignancy	• Biological: donor-derived **CIK** cells	• A O Papa Giovanni XXIII Bergamo, **Italy**
2	☐	Completed	Study of Adoptive Immunotherapy in Relapsed and Non-resectable Sarcomas After Multimodal Treatment.	• Sarcoma	• Drug: Autologous **CIK**	• AOU Città della Salute e della Scienza di Torino - Presidio Infantile Regina Margherita Turin, **Italy**
3	☐	Withdrawn	Study of Adoptive Immunotherapy in Relapsed and Non-resectable Sarcomas After Multimodal Treatment.	• Sarcoma	• Biological: Autologous **CIK** Dose level 1 • Biological: Autologous **CIK** Dose level 2 • Biological: Autologous **CIK** Dose level 3 • Biological: Autologous **CIK** Dose level 4	• Ospedale Infantile Regina Margherita - Unit of Paediatric Oncoematology Torino, **Italy**
4	☐	Completed Has Results	Cytokine Induced Killer (**CIK**) Cells In Leukemia Patients	• Hematologic Malignancies	• Biological: in vitro expanded Cytokine Induced Killer (**CIK**) cells	• Azienda Ospedaliera Papa Giovanni XXIII (Former:Ospedali Riuniti di Bergamo) Bergamo Bergamo, **Italy** • Ospedale Centrale di Bolzano Bolzano, **Italy** • Ospedale San Gerardo Monza, **Italy**
5	☐	Completed	Transposon-manipulated Allogeneic CARCIK-CD19 Cells in Pediatric and Adult Patients With r/r ALL Post HSCT	• Acute Lymphoblastic Leukemia, in Relapse	• Biological: CARCIK-CD19	• Ospedale PG23 Bergamo, BG, **Italy** • Fondazione MBBM Monza, MB, **Italy**

Background: CIK cell activity can be antigen-specifically retargeted via non-genetic approaches

A



B

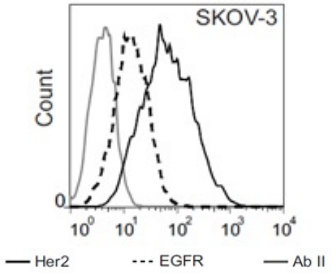


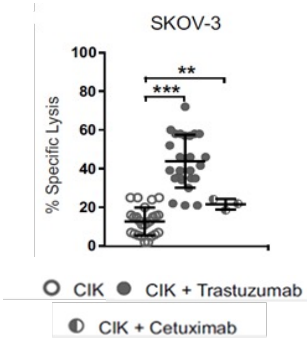
Cappuzzello *et al., Oncoimmunology 2016*

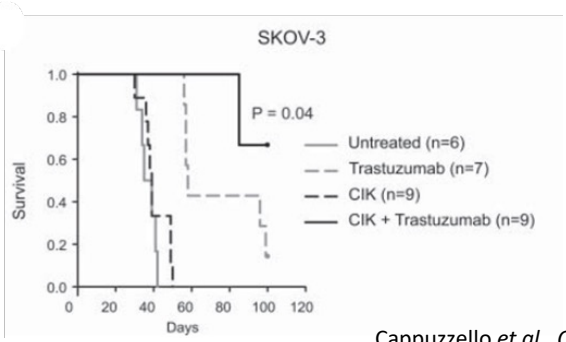
Clinical-grade mAb-mediated CIK cell retargeting in solid tumors

The combined therapy increases survival of NSG mice bearing ovarian or triple negative breast cancer (TNBC)

A — Ovarian cancer

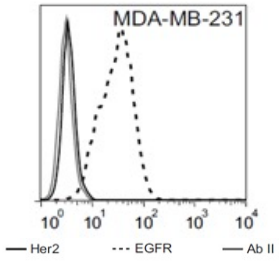


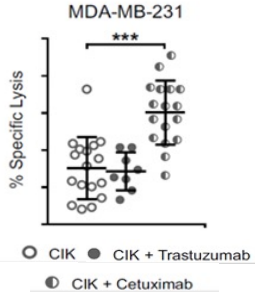


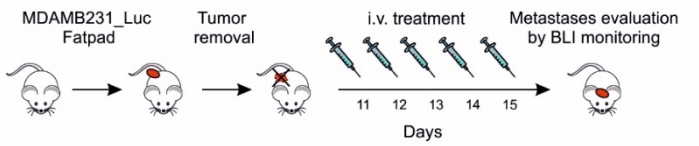


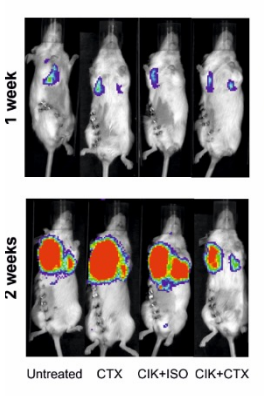
Cappuzzello *et al., Oncoimmunology 2016*

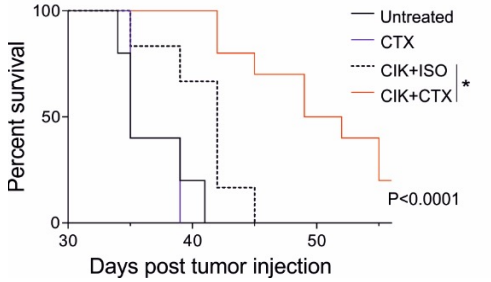
B — TNBC











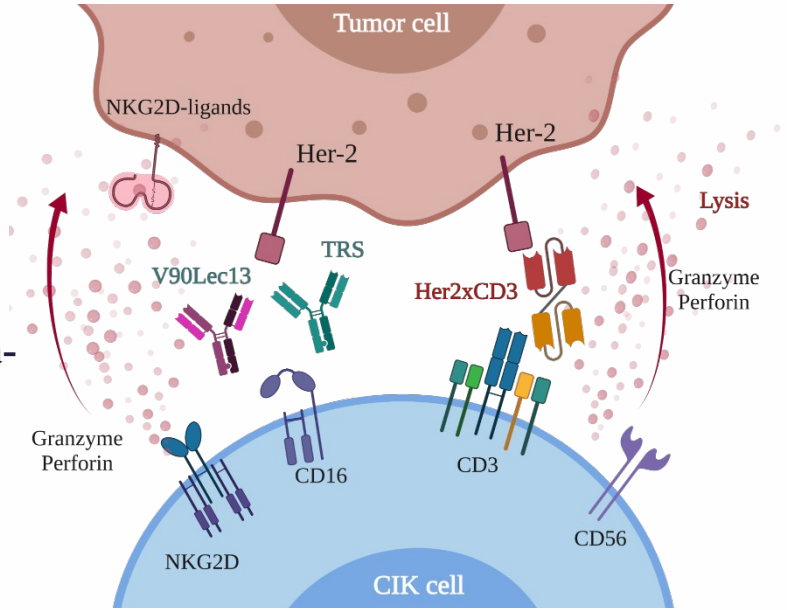
Sommaggio *et al., Oncoimmunology 2020*

Fc-optimized mAb or bsAb improve CIK cell retargeting against Her2^{+} breast cancer

Adoptive cell therapy with Cytokine-Induced Killer cells retargeted with immunotools against HER-2 expressing breast cancer

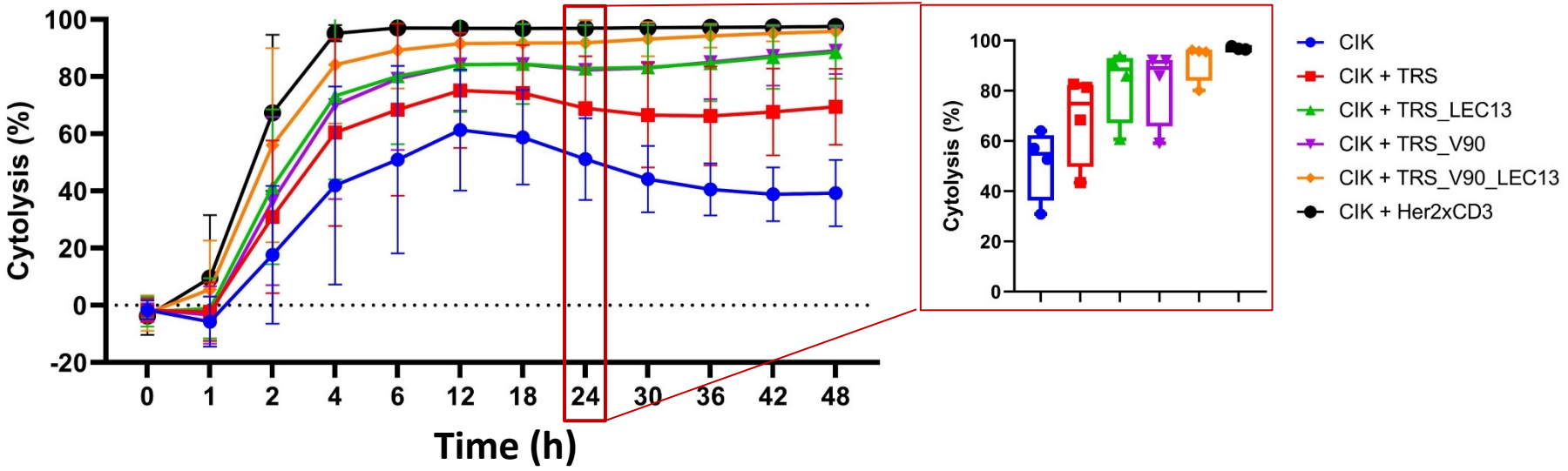
INNOVATIVE IMMUNOTOOLS

- Trastuzumab (TRS)
- TRS V90 – protein engineered
- TRS Lec13 – glyco engineered (a-fucosylated)
- TRS V90Lec13 – double engineered
- HER2xCD3 - bsAbs



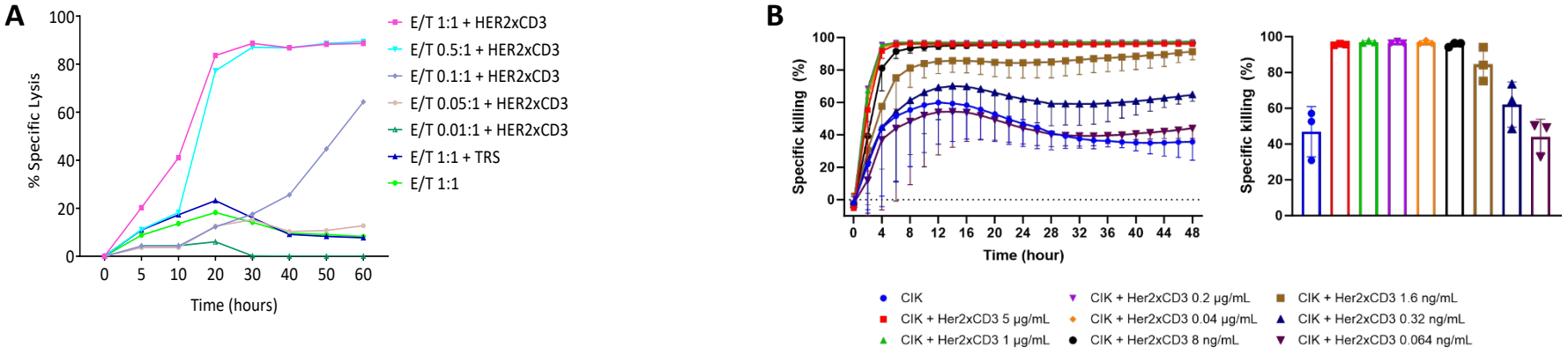
Fc-optimized mAb or bsAb improve CIK cell retargeting against Her2^{+} breast cancer (2)

Immunotools significantly increase killing of Her-2^{+} MCF-7 cell line by CIK cells

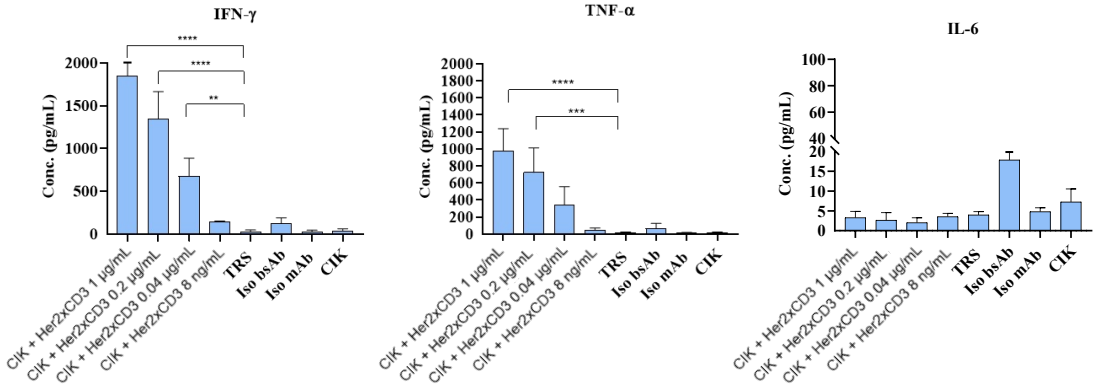


Fc-optimized mAb or bsAb improve CIK cell retargeting against Her2^{+} breast cancer (3)

CIK cells combined with Her2xCD3 rapidly kill Her-2^{+} target cells even at very low E/T ratios (A) and low dosage of bsAb (B)

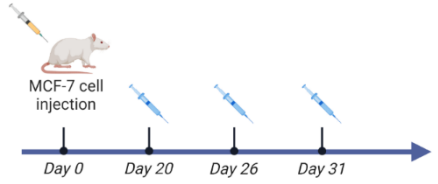


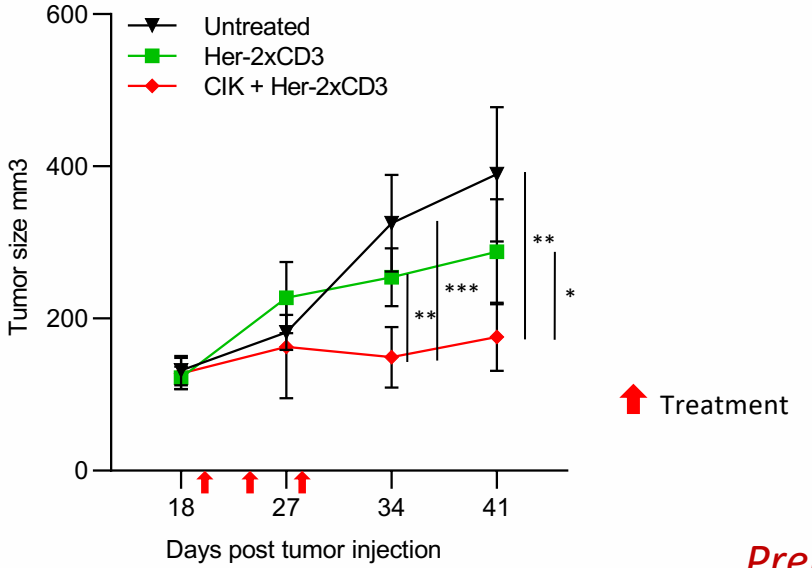
Her2xCD3-retargeted CIK cells maintain a safe pro-inflammatory cytokine profile



Fc-optimized mAb or bsAb improve CIK cell retargeting against Her2^{+} breast cancer (4)

CIK cells in combination with Her-2xCD3 delay tumor growth *in vivo*

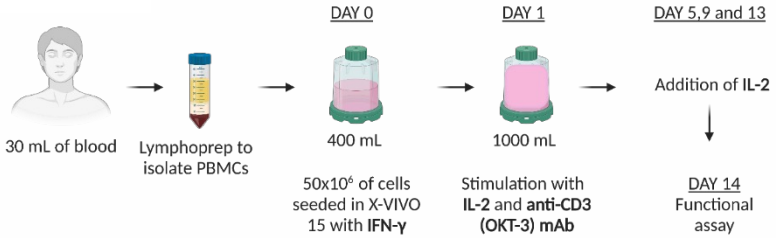




Preliminary experiments

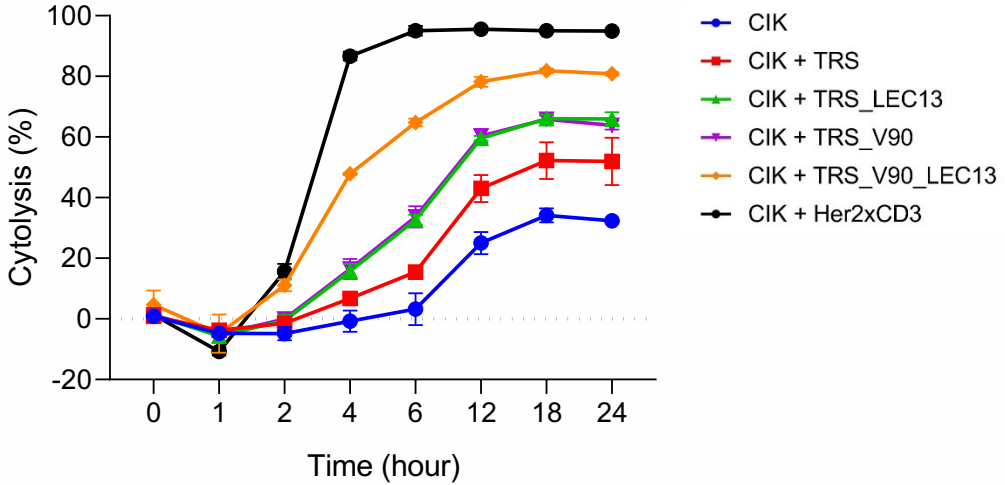
Fc-optimized mAb or bsAb improve CIK cell retargeting against Her2+ breast cancer (5)

Her2xCD3, TRS and engineered TRS significantly foster killing of Her-2+ MCF-7 cell line (E/T 10:1) by CIK cells from Her2+Breast patients



Small scale GMP- CIK cell expansion:

- from 5x10⁶ PBMC to 516x10⁶ CIK cells
- 103.2 fold increase



Clinical-grade mAb-mediated CIK cell retargeting in hematological malignancies

GMP-compliant protocol for CIK cell generation

Contents lists available at ScienceDirect

CYTOTHERAPY

journal homepage: www.isct-cytotherapy.org

International Society ISCT Cell & Gene Therapy

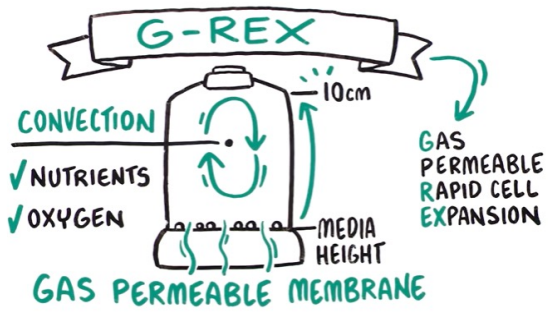
FULL-LENGTH ARTICLE

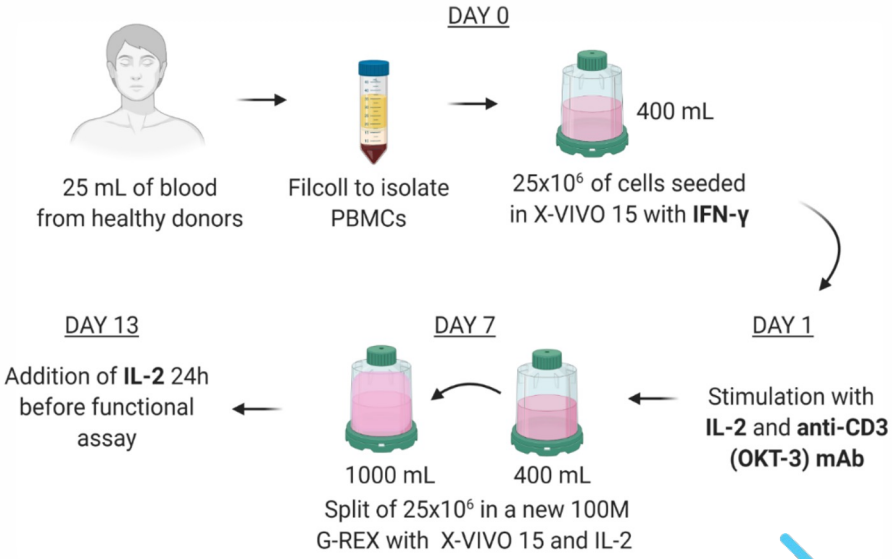
Manufacturing

A serum-free protocol for the *ex vivo* expansion of Cytokine-Induced Killer cells using gas-permeable static culture flasks

Pierangela Palmerini[1], Anna Dalla Pietà[1], Roberta Sommaggio[2], Annavera Ventura[1], Giuseppe Astori[3], Katia Chieregato[3], Maria Chiara Tisi[4], Carlo Visco[4], Omar Perbellini[4], Marco Ruggeri[4], Elisa Cappuzzello[1,*], Antonio Rosato[1,2,*,**]

[1] Department of Surgery, Oncology and Gastroenterology, Immunology and Oncology Section, University of Padua, Padua, Italy
[2] Veneto Institute of Oncology IOV – IRCCS, Padua, Italy
[3] Advanced Cellular Therapy Laboratory, Department of Hematology, Vicenza Hospital, Vicenza, Italy
[4] Hematology Department, San Bortolo Hospital, Vicenza, Italy

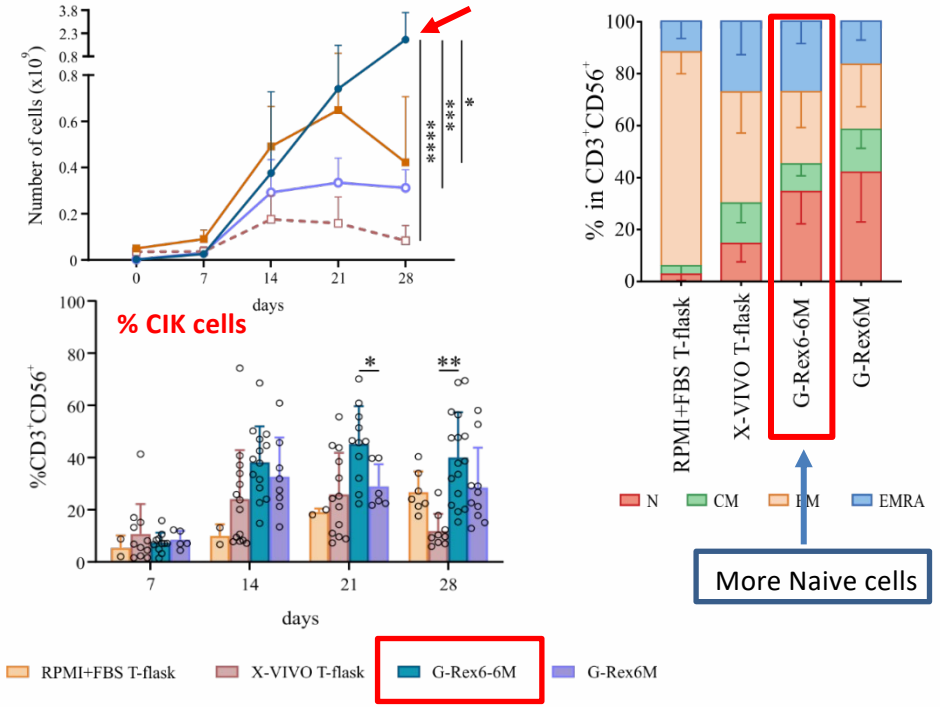




LTCA
LABORATORIO DI TERAPIE CELLULARI AVANZATE

GMP-compliant protocol for CIK cell generation

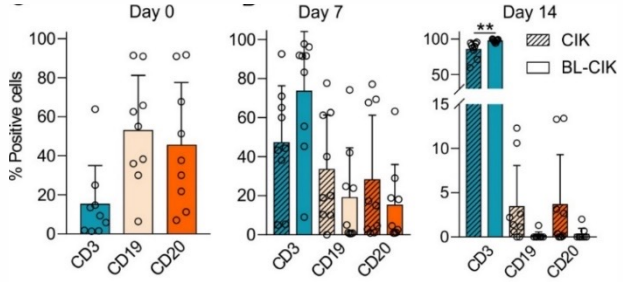
- This protocol dramatically reduces the culture manipulation and costs
- G-Rex® devices allow to obtain large amounts of $CD3^+CD56^+$ cells with high cytotoxic activity and a naïve phenotype.
- This strategy can be further and easily scalable to produce CIK cells for clinical applications.



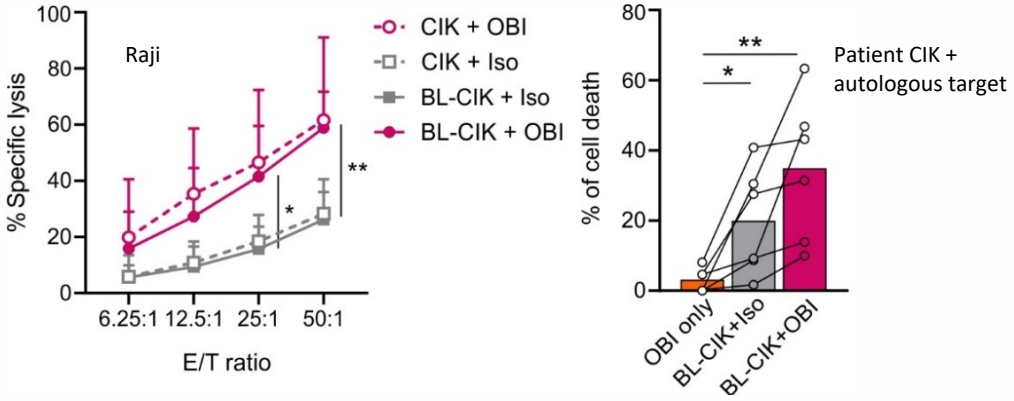
CIK cell retargeting against B-cell malignancies

Primary samples

→ The addition of Blinatumomab (CD19xCD3) eradicates residual malignant cells and improves CIK cell expansion



→ The addition of Blinatumomab does not impact CIK cell cytotoxicity in both allogeneic and autologous setting



Clinical-grade mAb-mediated CIK cell retargeting in B-cell malignancies

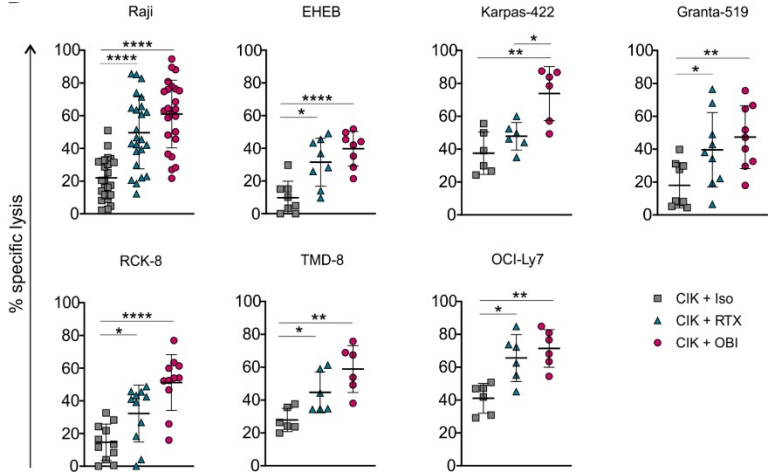
Anti-CD20 clinical-grade mAb efficiently redirect CIK cell activity against B-cell tumor lines and an aggressive patient-derived lymphoma xenograft (PDX)

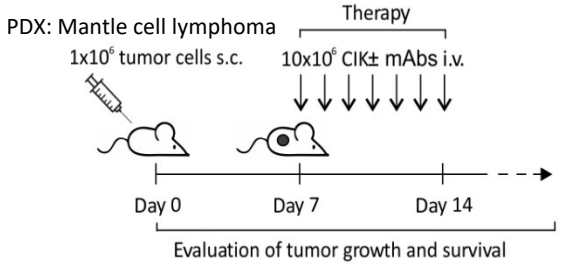
Open access Original research

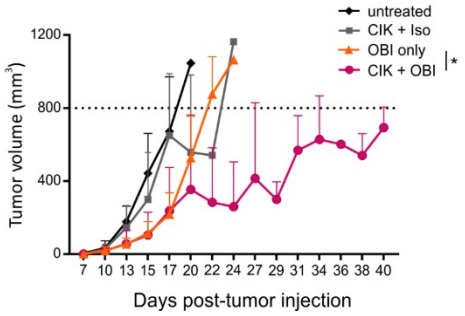
Journal for ImmunoTherapy of Cancer

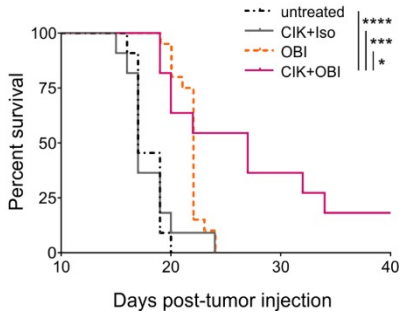
Innovative therapeutic strategy for B-cell malignancies that combines obinutuzumab and cytokine-induced killer cells

Anna Dalla Pietà,[1] Elisa Cappuzzello,[1] Pierangela Palmerini,[1] Annavera Ventura,[1] Andrea Visentin,[2] Giuseppe Astori,[3] Katia Chieregato,[3,4] Valentina Mozzo,[5] Omar Perbellini,[6] Maria Chiara Tisi,[6] Livio Trentin,[2] Carlo Visco,[7] Marco Ruggeri,[6] Roberta Sommaggio,[5] Antonio Rosato[1,5]

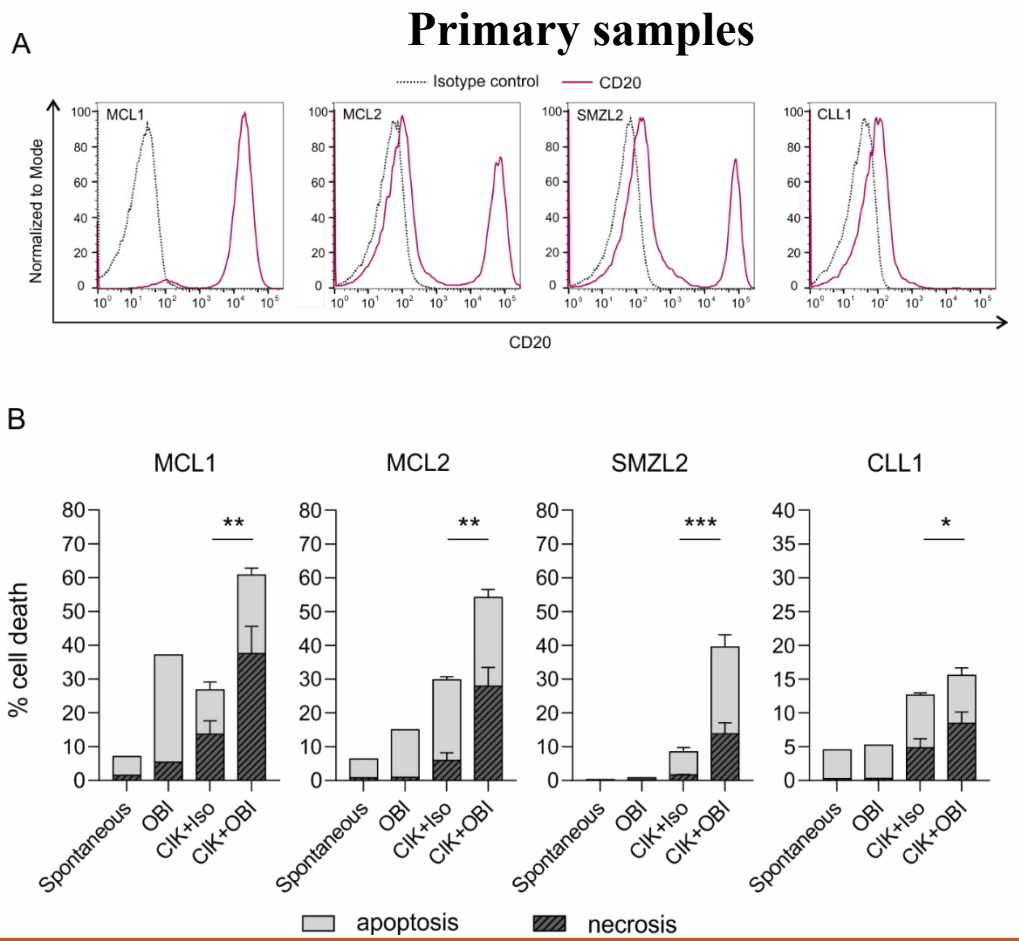








Anti-CD20 clinical-grade mAb efficiently redirect CIK cell activity against B-cell primary tumors from patients



CIK cell retargeting against B-cell malignancies - *clinics*

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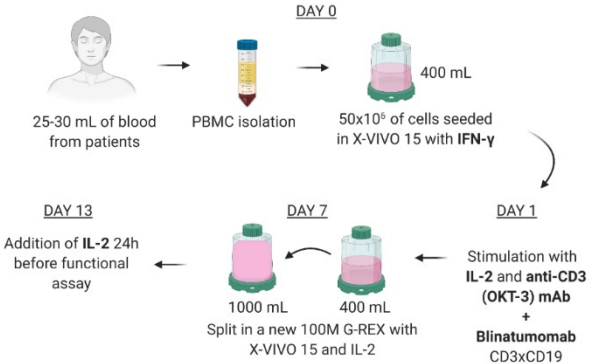
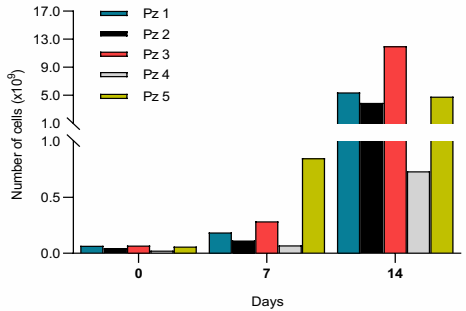
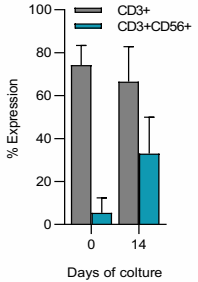
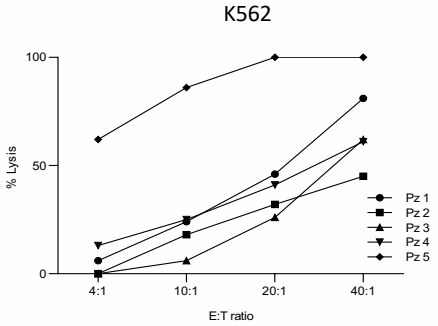
Generation and *ex vivo* expansion of CIK cells from DLBCL patients



AIFA AGENZIA ITALIANA DEL FARMACO



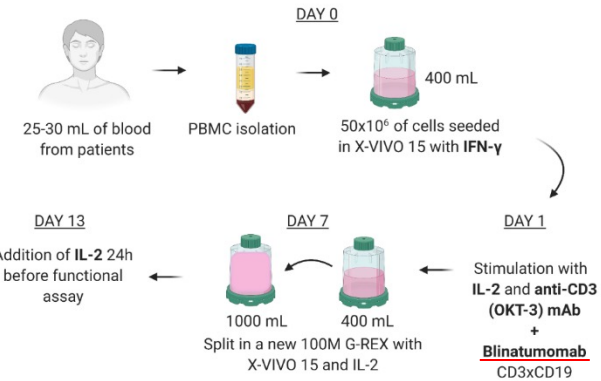


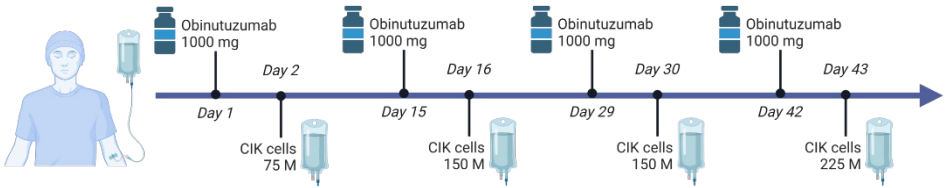


Clinical-grade mAb-mediated CIK cell retargeting in B-cell malignancies (2)

Autologous CIK cells combined with obinutuzumab: First report on clinical feasibility

- 59 years old woman
- diffuse large B-cell lymphoma (DLBCL)
- relapsing after four lines of therapy
  - rituximab with chemotherapy (R-DA-EPOCH, R-DHAOX)
  - polatuzumab vedotin
  - CAR-T tisagenlecleucel (OSS)
- 20 ml of peripheral blood (no apheresis, collection after 1st line)
- four infusions of escalating doses of CIK cells (from 1 x 10^6/kg to 3 x 10^6/kg)

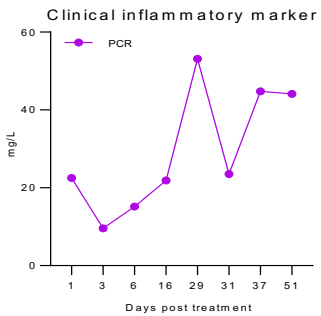


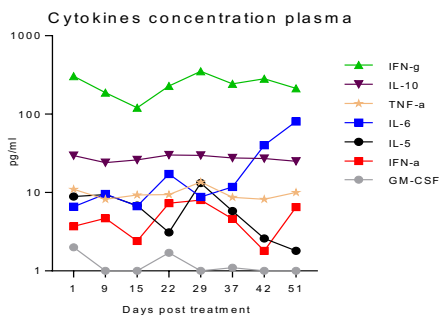


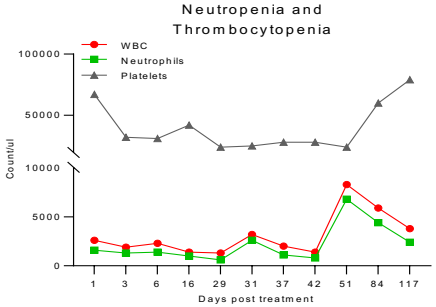
Elice *et al.*, ISTC Paris 2023, Poster 1023 *Immunotherapy*

Clinical-grade mAb-mediated CIK cell retargeting in B-cell malignancies (3)

Autologous CIK cells combined with obinutuzumab:
First report on clinical feasibility (2)



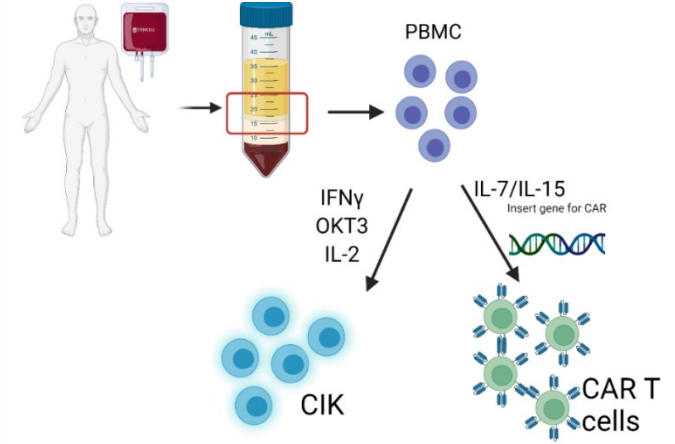


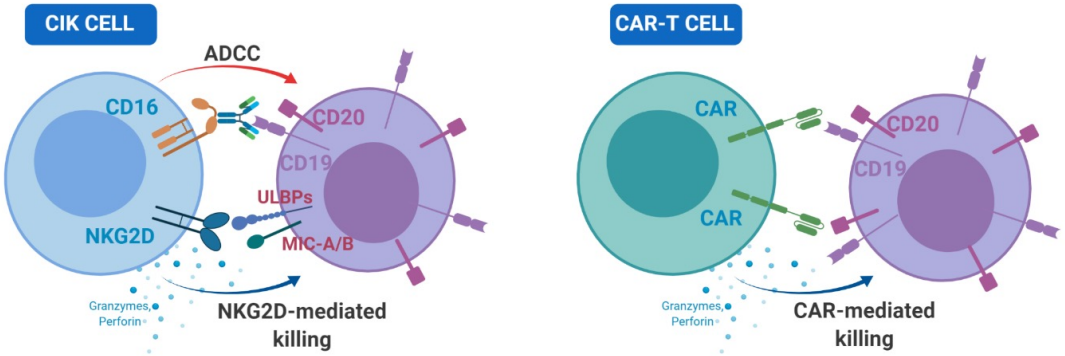


- Absence of infusional reactions, cytokine release syndrome, neurotoxicity
- Transient reduction of platelet count after Obi
- PET scan evaluation: decrease in the number of involved sites with a unique stable residual node

This therapy allowed to arrest the progression of the disease for 9 months (to date) without infectious or inflammatory complications, and was used as a **bridge to allogeneic transplantation.**

What role for retargeted CIK cells vs CAR-T cells?

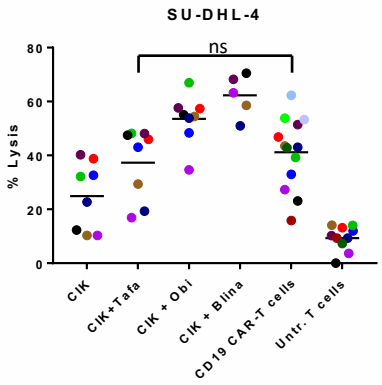




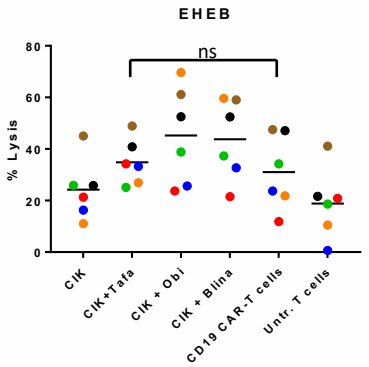
mAb or bsAb-retargeted CIK cells essentially equal CAR-T cells *in vitro*

Redirected CIK and CAR-T cells from healthy donors challenged against CD19⁺/20⁺ tumor cell lines

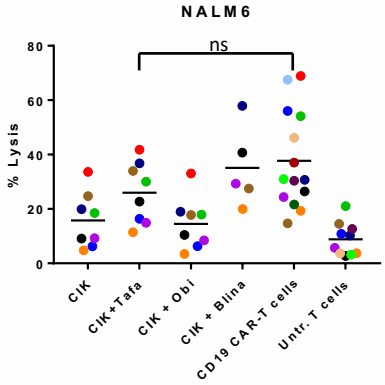
Diffuse Large B-cell lymphoma (DLBCL)



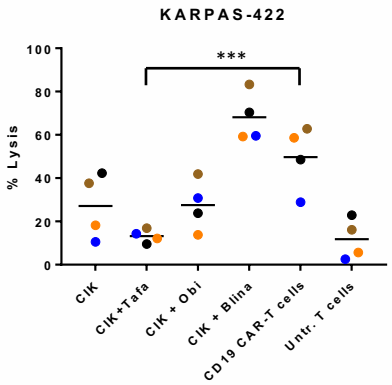
Chronic lymphocytic leukemia (B-CLL)



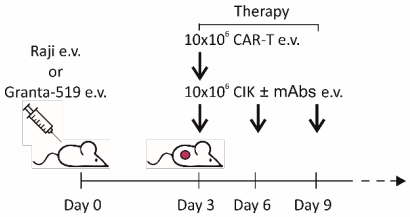
Acute lymphoblastic leukemia (ALL)



Follicular lymphoma (FL)



Retargeted CIK cells may represent a valuable therapeutic option



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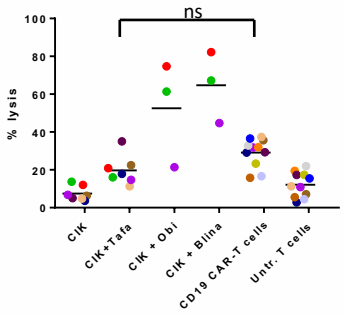

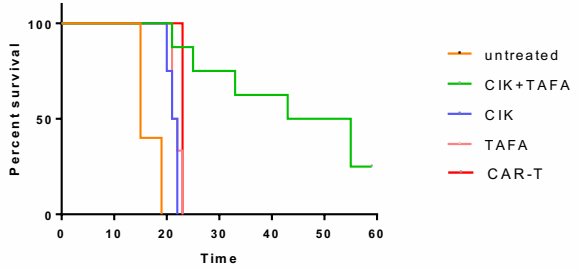

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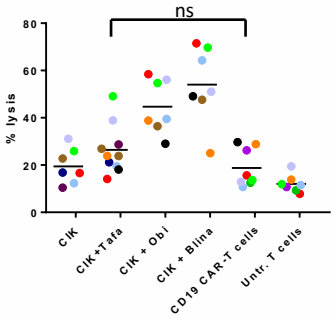
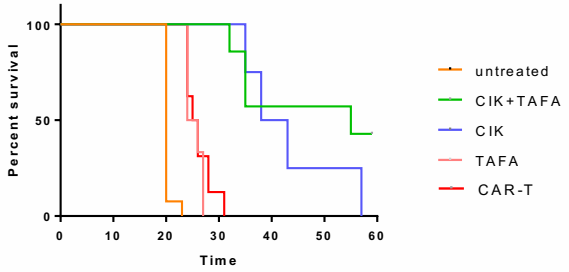
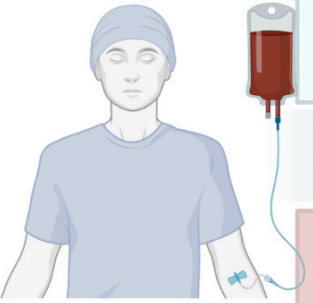






Overall **high** costs

PITFALLS of CAR-T manufacturing

Large amount of blood required

Very few authorized cell factories worldwide

Complex and expensive

Expensive devices; one batch at a time; large amount of medium required; inefficient for heavely pre-treated patients

Cost increase and long waiting time

Leukapheresis → Shipping → Activation → Gene transfer → Expansion → Cryoconservation and delivery

Small volume of peripheral or cord blood

Academic GMP facilities

Not required

Easy to use devices (G-Rex); more batches at a time; **efficient static** cell growth from potentially every patient type

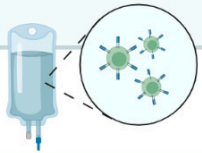
Locally available

Reduced overall costs

CIK cells as a potential alternative

PATIENT INFUSION

- Complex patient care needs and follow-up only in reference centers
- Risk of treatment-related toxicities (CRS, ICANS) and relapse
- Challenging shift to allogeneic setting



- Manageable mild AEs eventually in outpatient setting
- Potential implementation as bridge therapy
- Multiple infusions to tailor the therapy
- Reduced GvHD even in allogeneic setting

Conclusions

- We envisage a **"universal" platform for ACT**, where the effectors are represented by a sole subset, CIK cells, easy to generate and expand at low cost and already endowed with strong cytotoxicity and potential therapeutic activity, which can be further improved *à la carte* by antigen-specific retargeting with different immunotools depending on the tumor histotype and antigen expression, without any need to genetically modify the cells.

- Since several of such immunotools are already used in patients, the clinical translation of the combined approach is just around the corner.

A hope for CIK cell therapy

Now

Future

Acknowledgements

University of Padova
Veneto Institute of Oncology – IRCCS

Prof. Antonio Rosato

Roberta Sommaggio
Elisa Cappuzzello
Anna Dalla Pietà
Giulia D'Accardio
Emilia Vigolo
Hieu Trong Ngo

Debora Carpanese
Silvia Dalla Santa
Beatrice Genova
Alessandro Penna
Valentina Rossi
Sara Santi
Anna Tosi
Gaia Zuccolotto

Sara Perpinello
Annavera Ventura

Gaia Griguolo

LTCA – Laboratorio di Terapie Cellulari Avanzate, Vicenza
Giuseppe Astori & Team

Ospedale S. Bortolo, AULSS 8 Berica
Alberto Tosetto
Francesca Elice
Marco Ruggeri
Maria Chiara Tisi
Marcello Riva

University of Kiel, Germany
Prof. Matthias Peipp
Christian Kellner

University of Verona
Carlo Visco
Mauro Krampera

University of Padova
Livio Trentin
Andrea Visentin