CAR-T cells: basics, applications and data reporting

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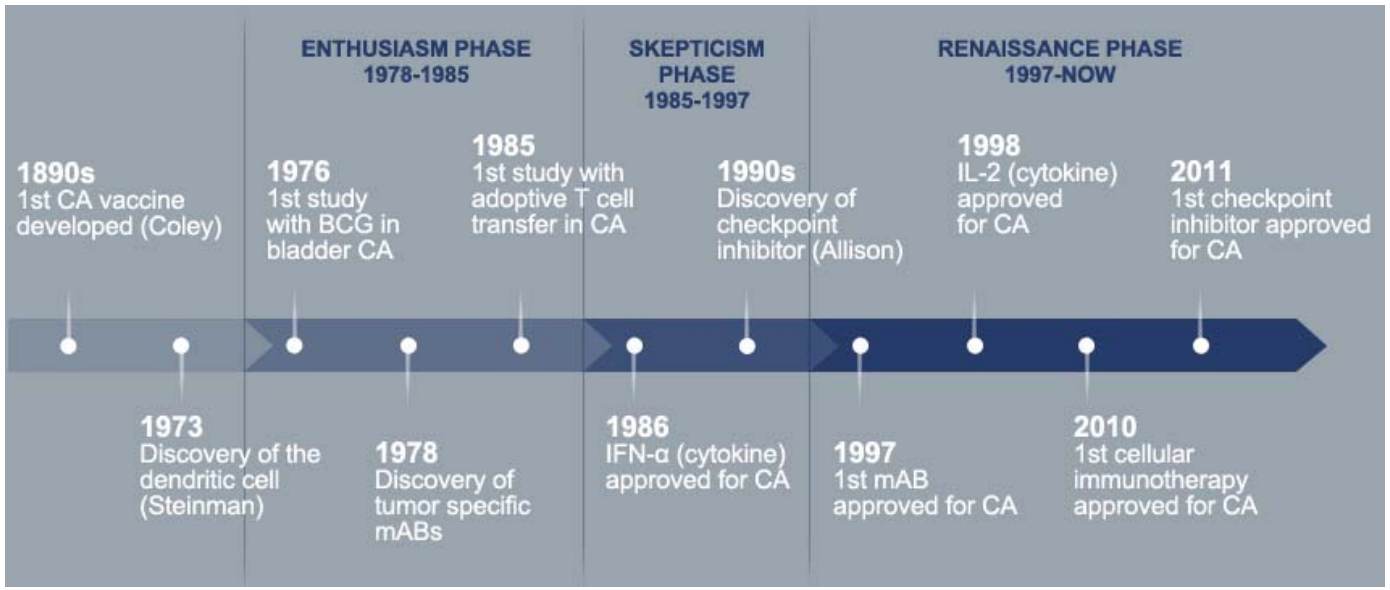
2011

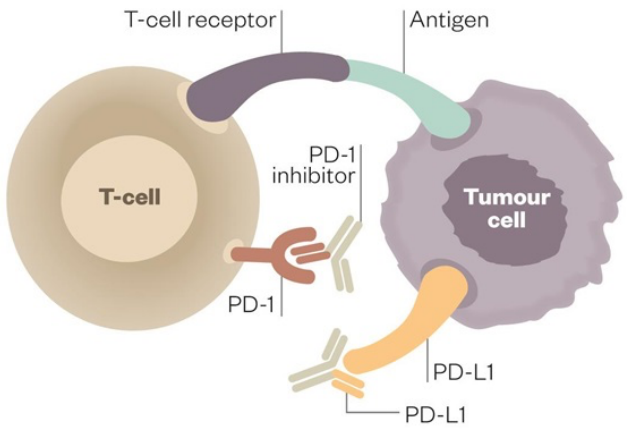
2012

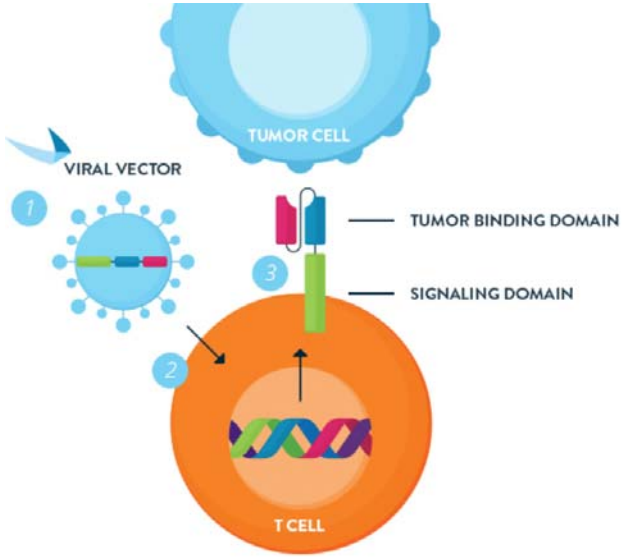

NO ONE
FIGHTS
ALONE.

Breakthrough of the Year 2013



Immune checkpoint blockade and engineered T cells are two radically different approaches to cancer immunotherapy

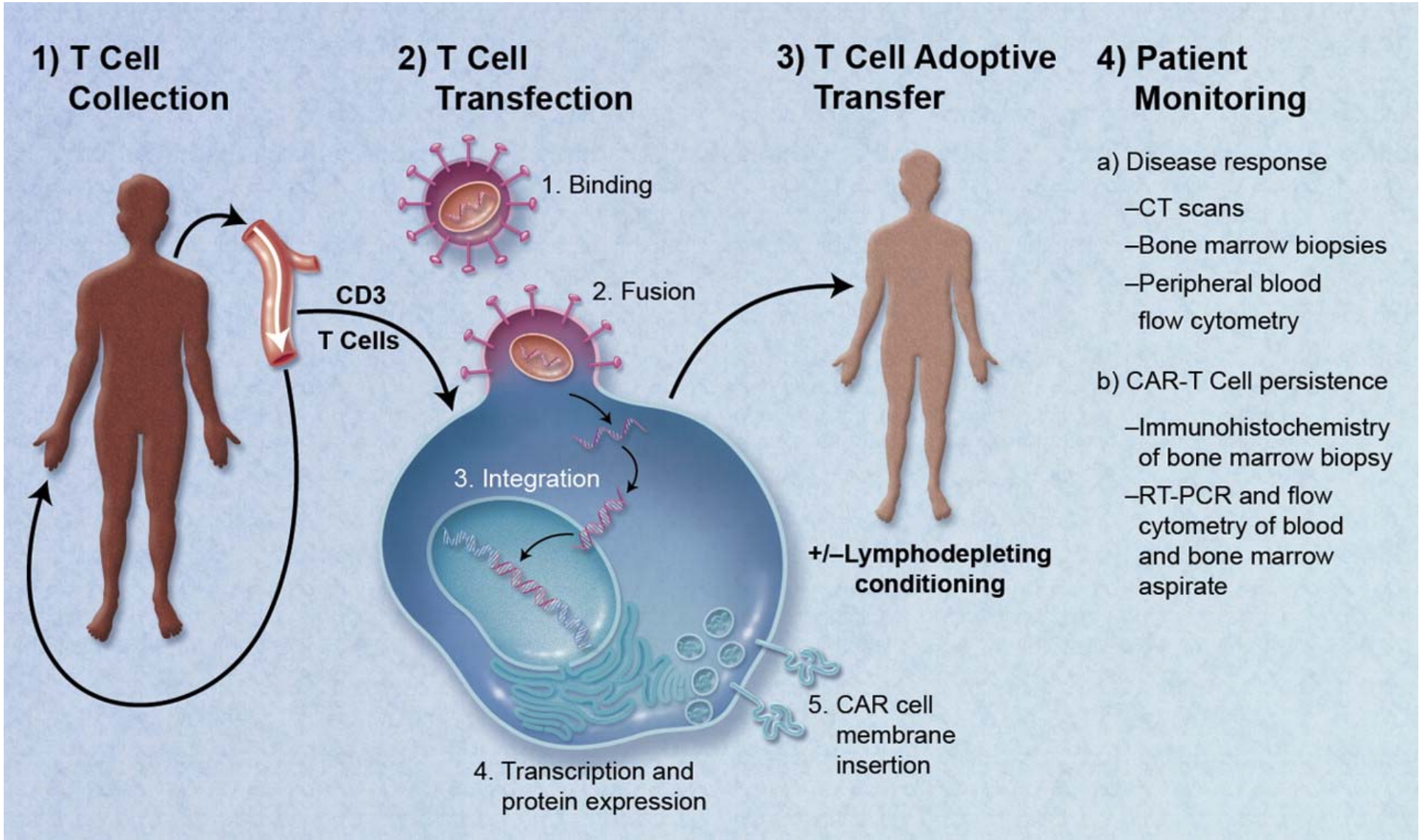




Bingo approach
Pre-existing immunity
Multiple tumor indications
Off-the-shelf

Designer approach
Overcomes tolerant repertoire
Single tumor indications
Highly personalized

Engineered T cells are the most powerful anti-tumor effectors you can dream of


1) T Cell Collection
2) T Cell Transfection
3) T Cell Adoptive Transfer
4) Patient Monitoring
1. Binding
2. Fusion
CD3
T Cells
3. Integration
+/–Lymphodepleting conditioning
5. CAR cell membrane insertion
4. Transcription and protein expression
a) Disease response
–CT scans
–Bone marrow biopsies
–Peripheral blood flow cytometry
b) CAR-T Cell persistence
–Immunohistochemistry of bone marrow biopsy
–RT-PCR and flow cytometry of blood and bone marrow aspirate

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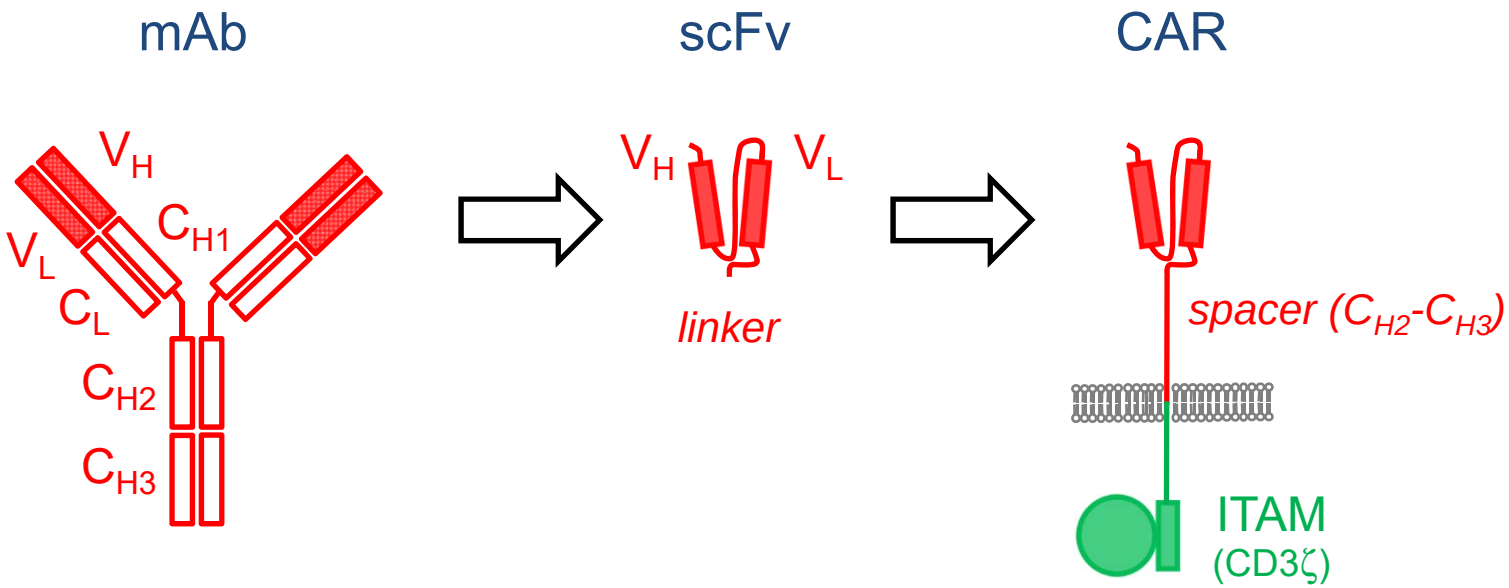
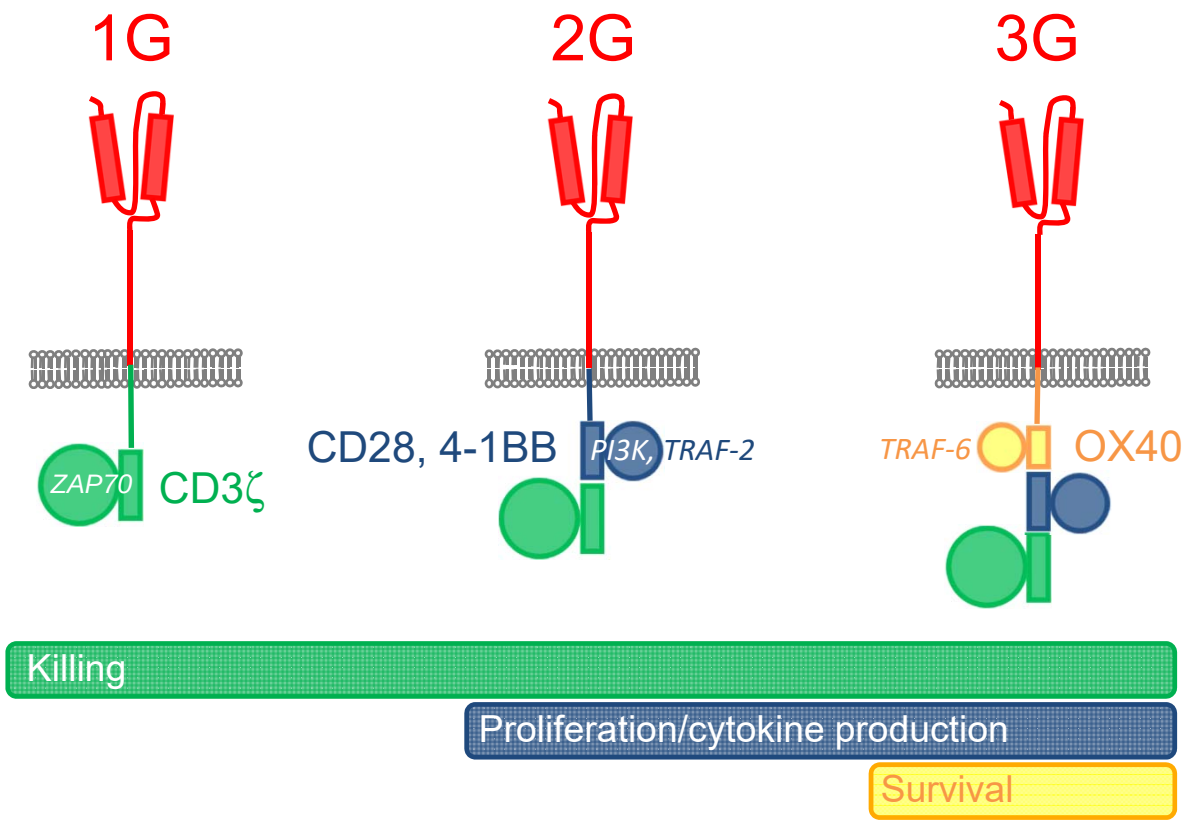
CARs are *Frankenstein* molecules made up of an mAb-derived antigen-binding moiety and a TCR-derived signaling domain



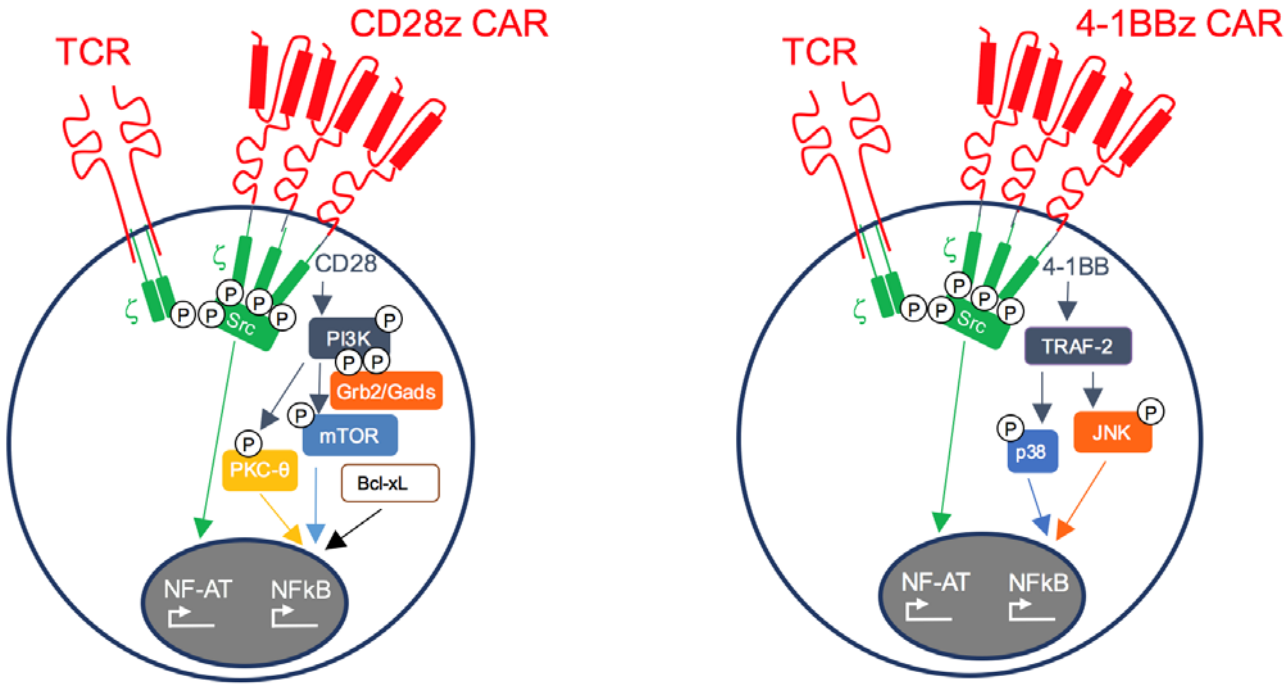
TCRs
HLA-dependent
Intracellular Ags
Protein Ags
Low affinity (10^{-3}-10^{-4})
Killing and proliferation

CARs
HLA-independent
Surface Ags
Protein, sugar and lipid Ags
High affinity (10^{-8}-10^{-10})
Killing

CARs are highly *modular* receptors that can fit additional signaling domains



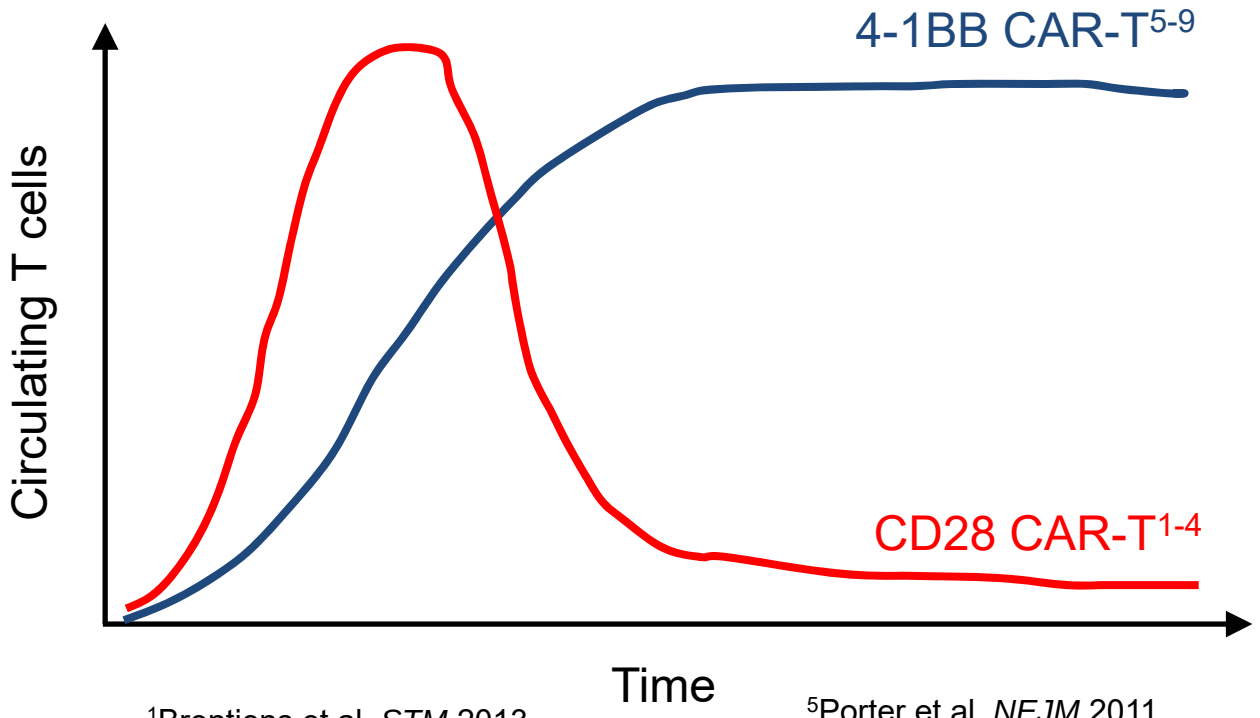
CD28 and 4-1BB differently affect the pharmacodynamics (PD) of CAR-T cells



CD28
PI3K
Anti-apoptotic
Glycolysis

4-1BB
TRAF-2
Pro-apoptotic?
Fatty acid oxydation

CD28 and 4-1BB differently affect the pharmacokinetics (PK) of CAR-T cells



[1]Brentjens et al, *STM* 2013
[2]Kochenderfer et al, *JCO* 2014
[3]Lee et al, *Lancet Oncol* 2015
[4]Wang et al, *Blood* 2016

[5]Porter et al, *NEJM* 2011
[6]Porter et al, *STM* 2011
[7]Grupp et al, *NEJM* 2013
[8]Turtle et al, *JCI* 2015
[9]Maus et al, *NEJM* 2014

H2020 CARAT: automated systems for robust, scalable and sustainable CAR-T cell manufacturing

CARAT *vs* operator
Closed system
Automated process
Flexible (electroporator, FACS)
May work in assembly lines

The future of CAR-T cell manufacturing will likely move to the point-of-care

2010s

2020s

2030s

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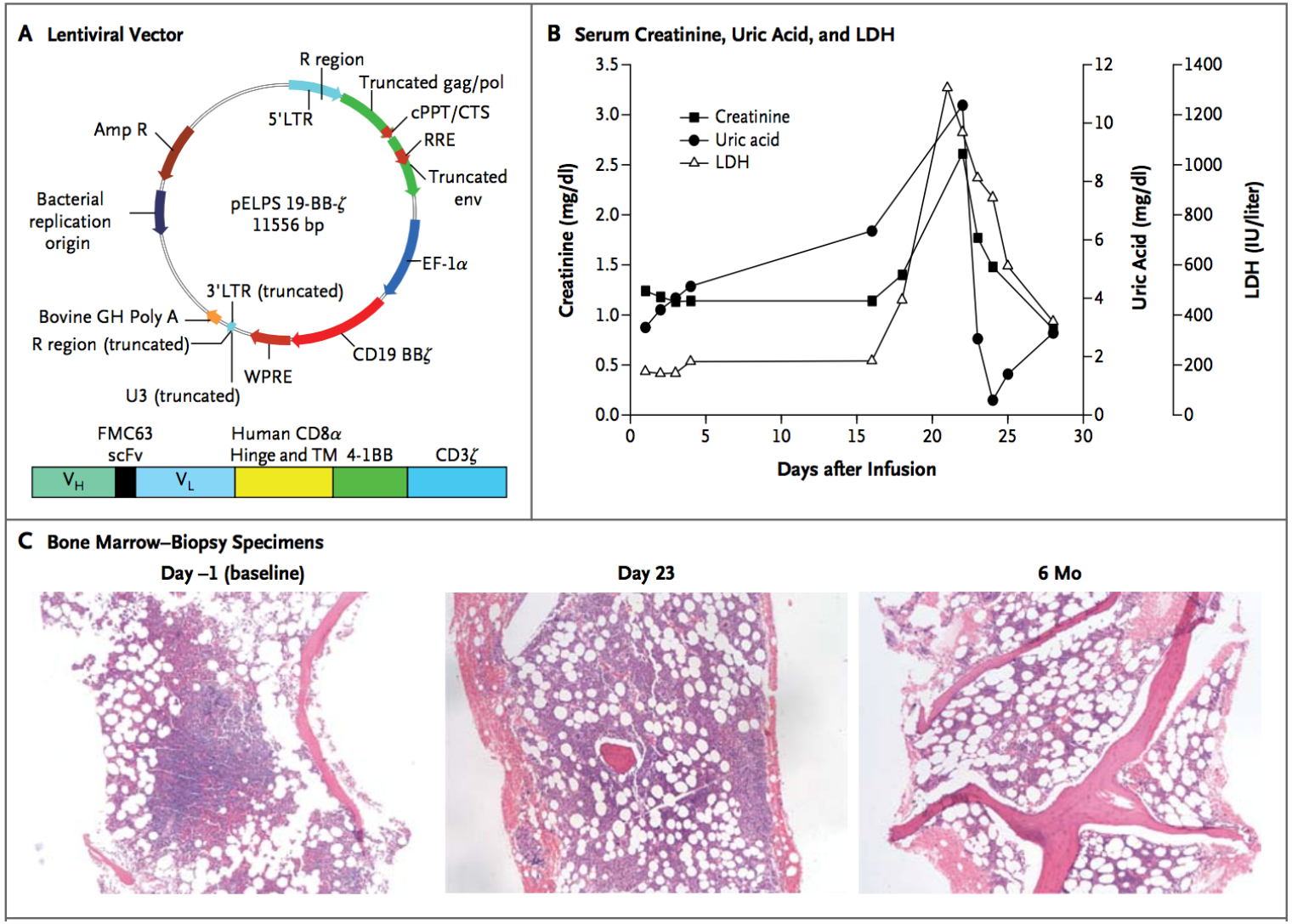
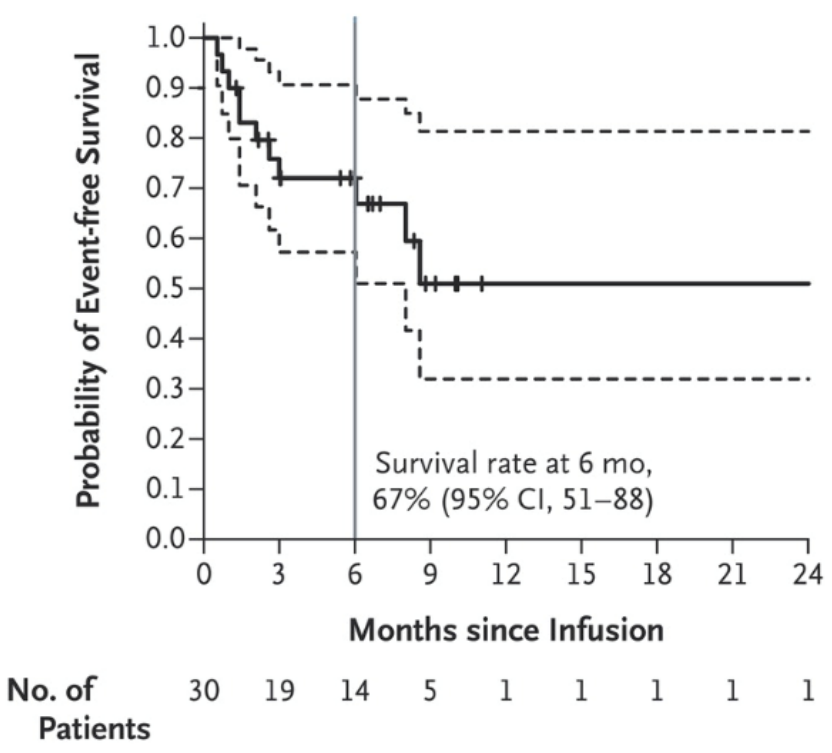
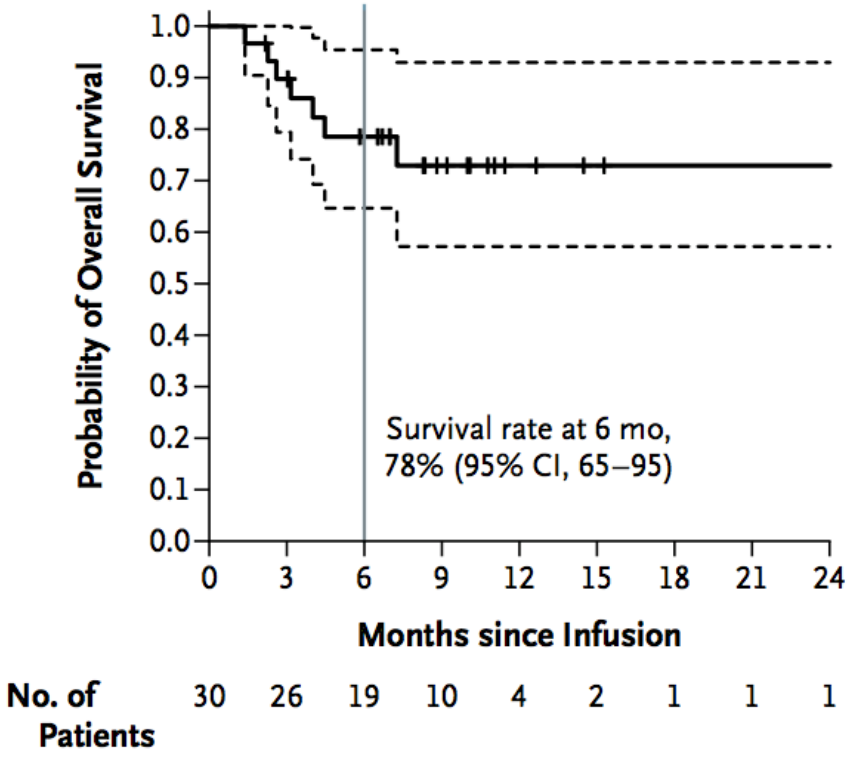
It all began with anecdotal impressive clinical results of CD19 CAR-T cells in CLL



Porter et al, *NEJM* 2011

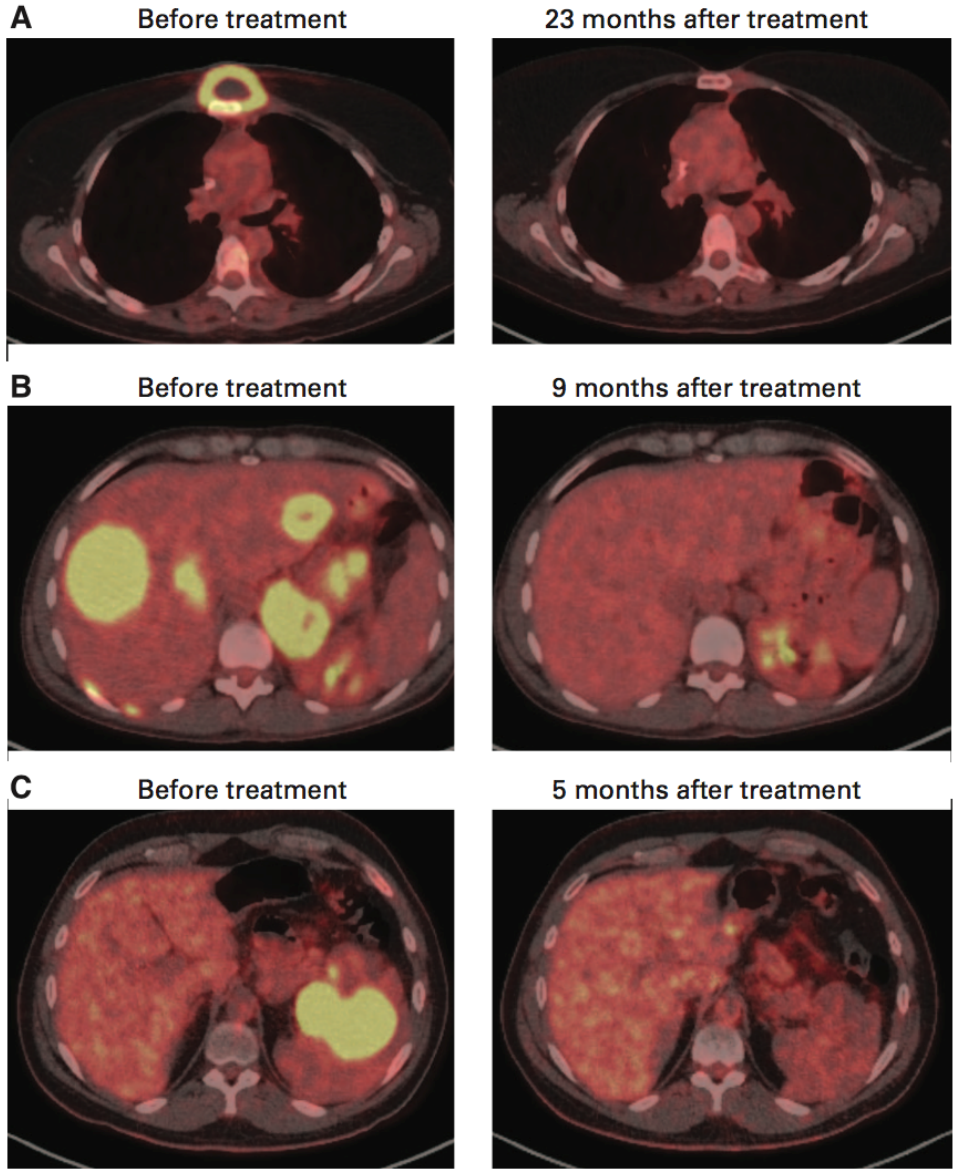
CD19 CAR-T cells achieve up to 90% CR in R/R B-ALL





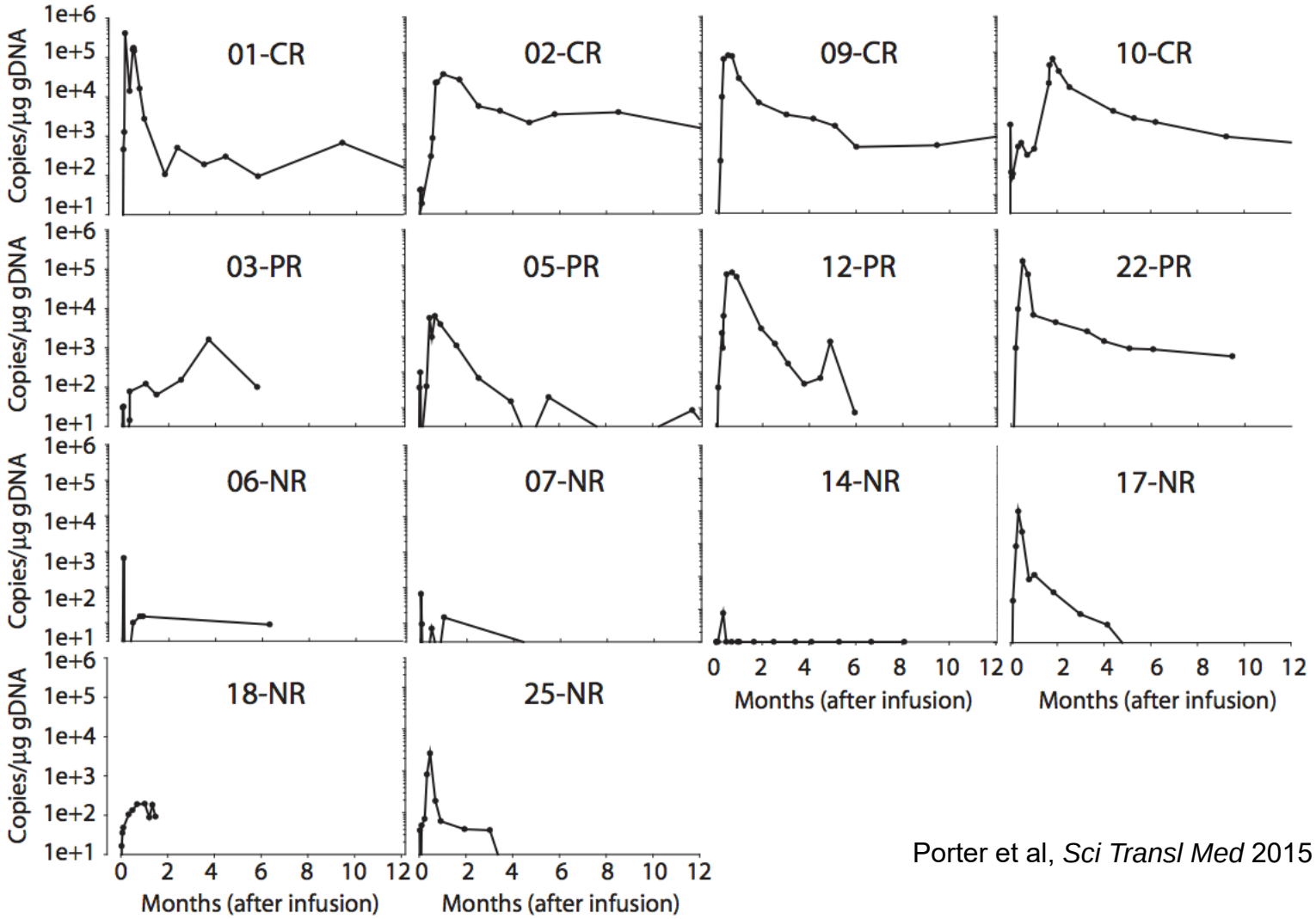
Maude et al, *NEJM* 2014

CD19 CAR-T cells show promising signs of efficacy in DLBCL



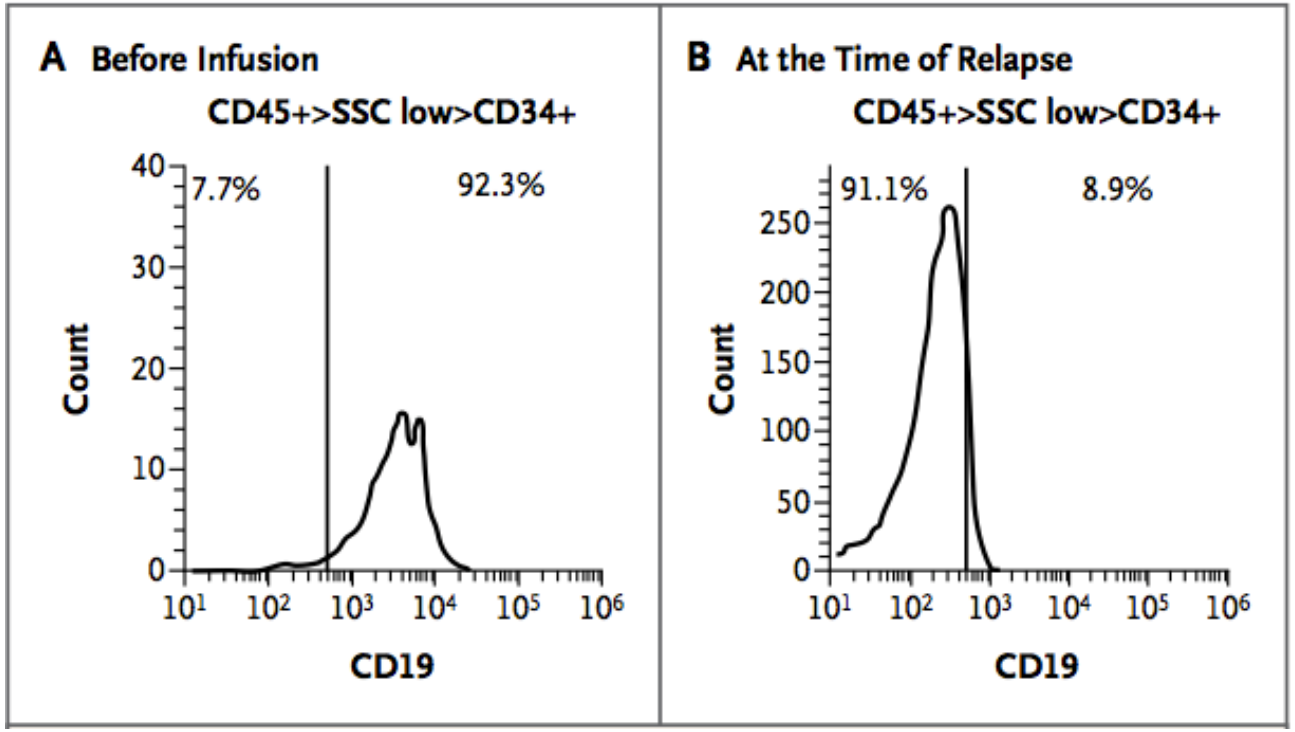
Kochenderfer et al, *JCO* 2014

Antitumor responses are highly variable and correlate with CAR-T cell engraftment



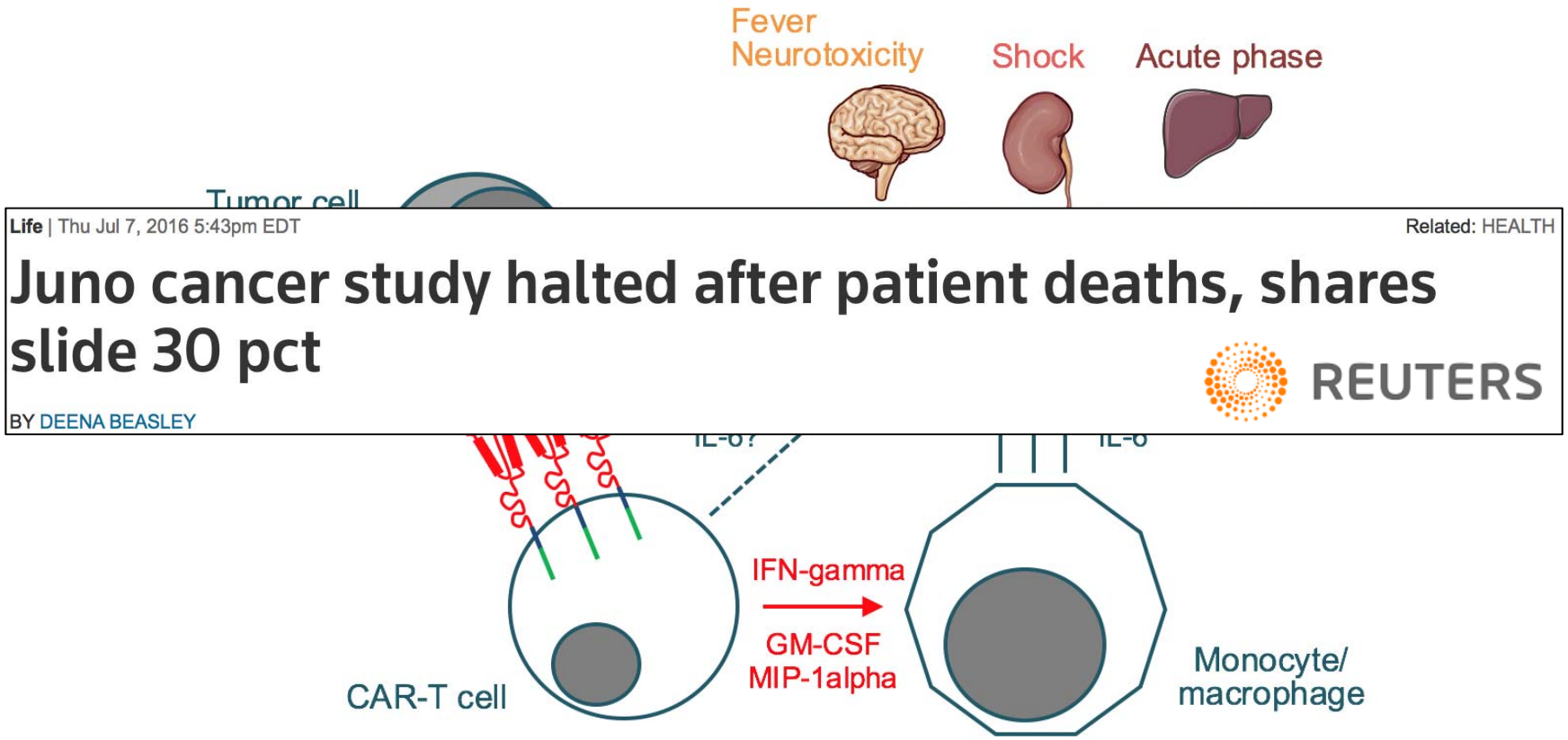
Porter et al, *Sci Transl Med* 2015

The genomic loss of CD19 is a formidable secondary resistance mechanism to CAR-T cells



Maus et al, *NEJM* 2014

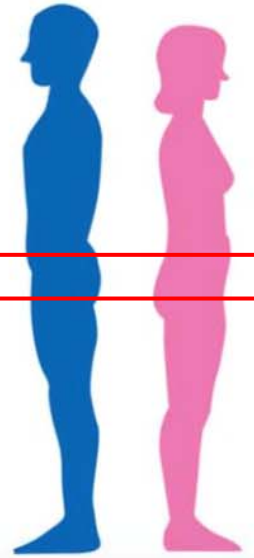
The cytokine release syndrome is a common and potentially lethal toxicity of CD19 CAR-T cells



A projected drop in mortality by CD19 CAR-T cells will only minimally impact on overall cancer mortality

Estimated Deaths

Males			Females		
Lung & bronchus	86,380	28%	Lung & bronchus	71,660	26%
Prostate	27,540	9%	Breast	40,290	15%
Colon & rectum	26,100	8%	Colon & rectum	23,600	9%
Pancreas	20,710	7%	Pancreas	19,850	7%
Liver & intrahepatic bile duct	17,030	5%	Ovary	14,180	5%
Leukemia	14,210	5%	Leukemia	10,240	4%
Esophagus	12,600	4%	Uterine corpus	10,170	4%
Urinary bladder	11,510	4%	Non-Hodgkin lymphoma	8,310	3%
Non-Hodgkin lymphoma	11,480	4%	Liver & intrahepatic bile duct	7,520	3%
Kidney & renal pelvis	9,070	3%	Brain & other nervous system	6,380	2%
All Sites	**312,150**	**100%**	**All Sites**	**277,280**	**100%**



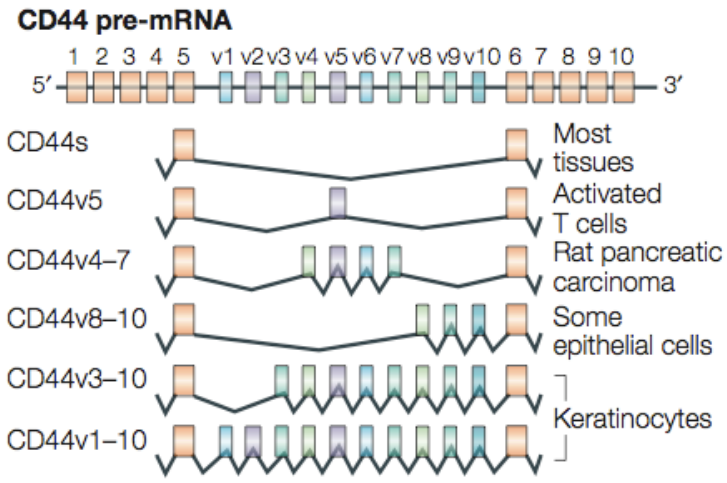
Projected mortality drop

Leukemia (2/3)	10,000
Lymphoma (1/2)	6,000
TOTAL	16,000 (<5%)

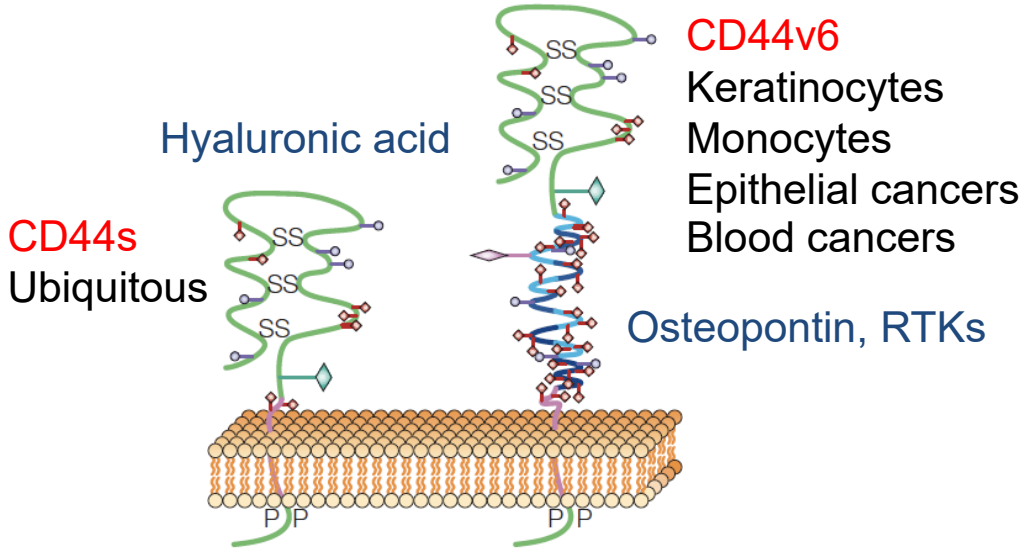
Leukemia (2/3)	7,000
NHL (1/2)	4,000
TOTAL	11,000 (<4%)

The choice of the CAR target antigen is possibly the single most important determinant of success

Type	Drawbacks
Lineage-restricted (CD19, BCMA)	Narrow tumor indications
Over-expressed (EGFR, mesothelin)	Off-tumor toxicity
Differently spliced (CD44v6)	Off-tumor toxicity (limited)
Differently glycosylated (mucin, CD44v6)	?
Cytoplasmic proteins (RHAMM)	?

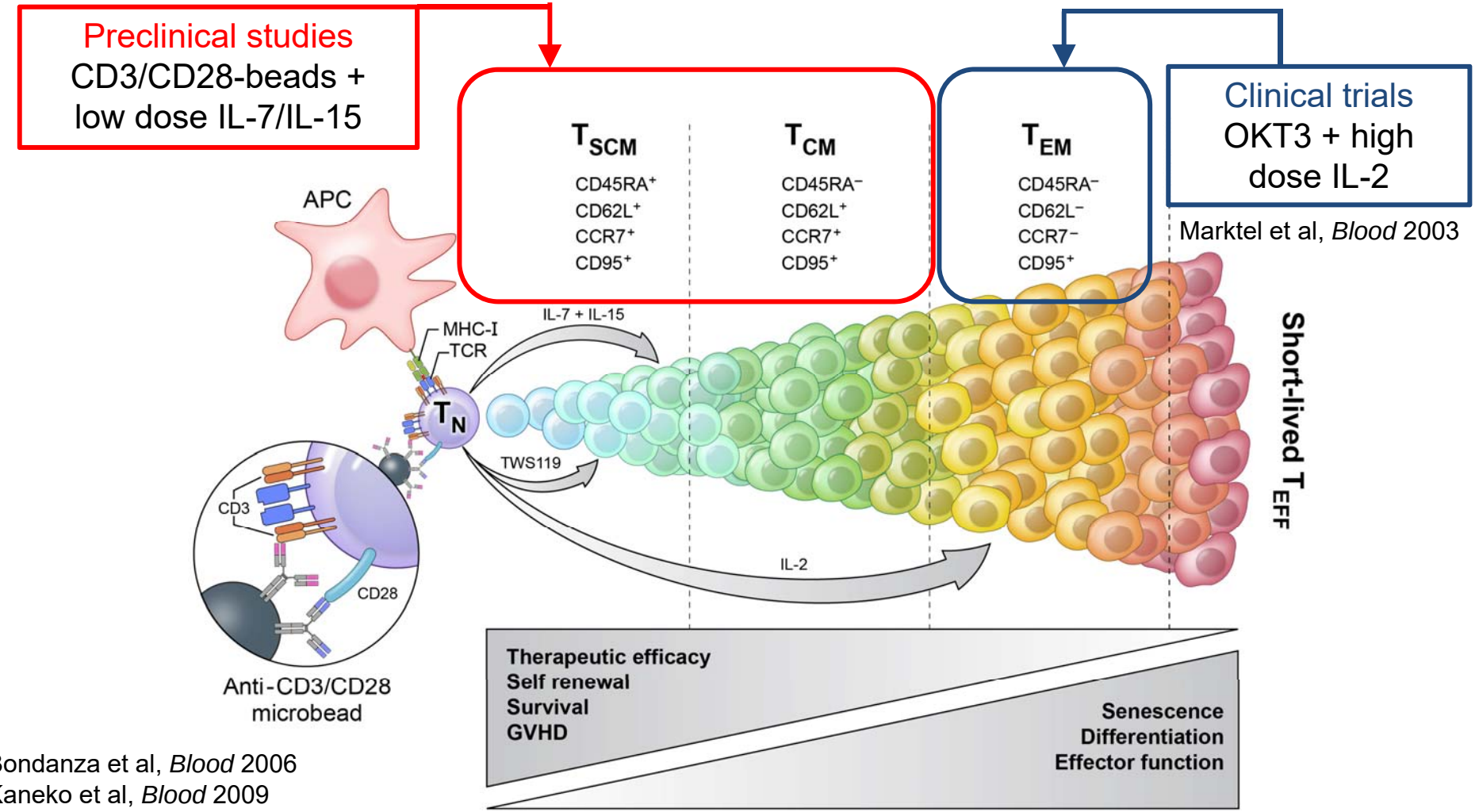


Maps on 11p, MW 37Kd (341 aa)



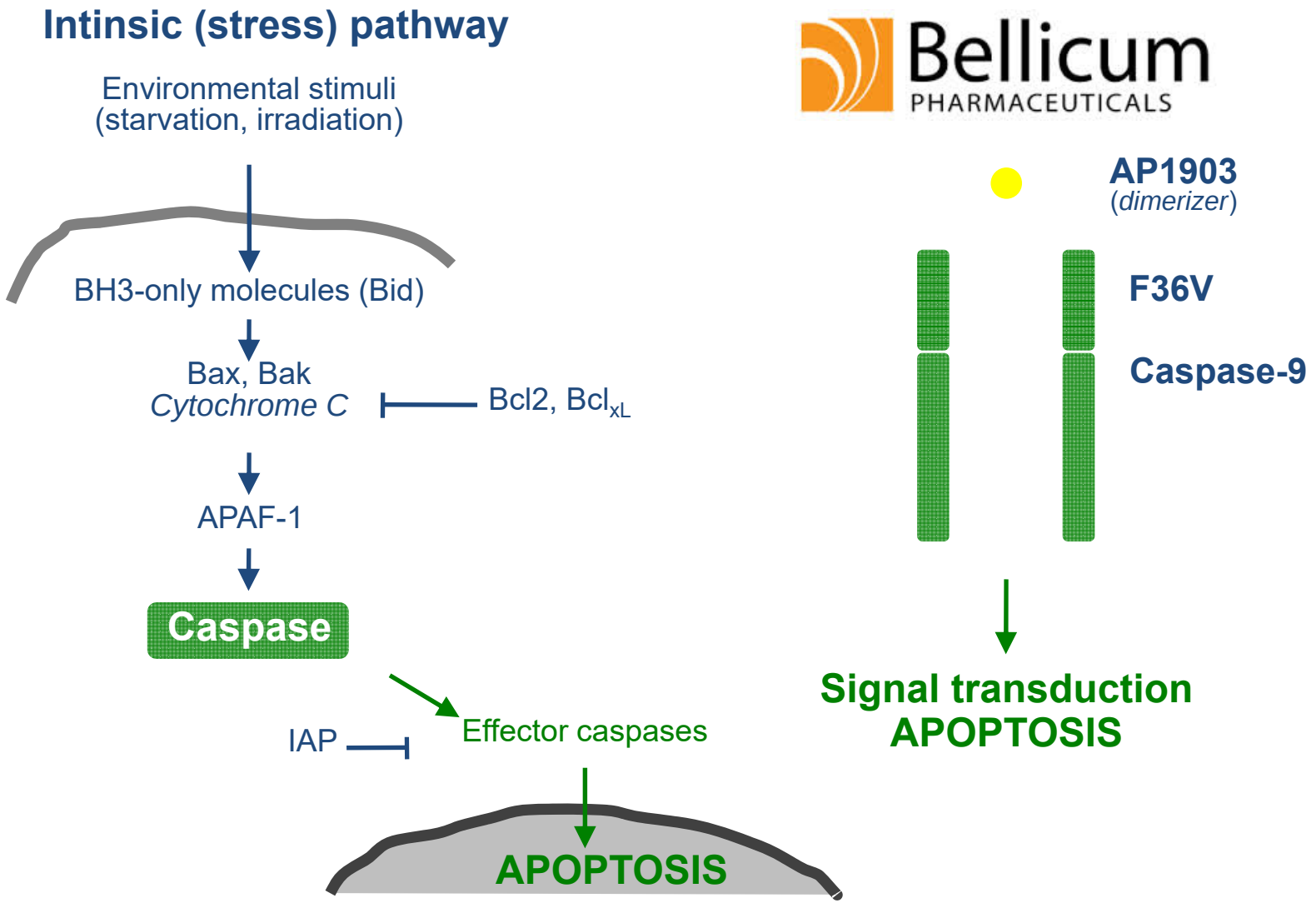
Merlin, Rho GTPases, Erk1

CAR-T cell manufacturing with CD3/CD28-beads and IL-7/IL-5 imprints an early-differentiation phenotype

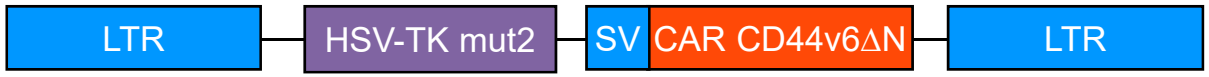


Bondanza et al, *Blood* 2006
Kaneko et al, *Blood* 2009
Bondanza et al, *Blood* 2011
Bondanza EP1946080 & US13240,009
Cieri et al, *Blood* 2013

Inducible caspase-9 is an innovative, fully humanized suicide gene



A phase I/IIa clinical trial of anti-CD44v6 CAR-T cells in relapsed/refractory AML and MM will begin in 2018

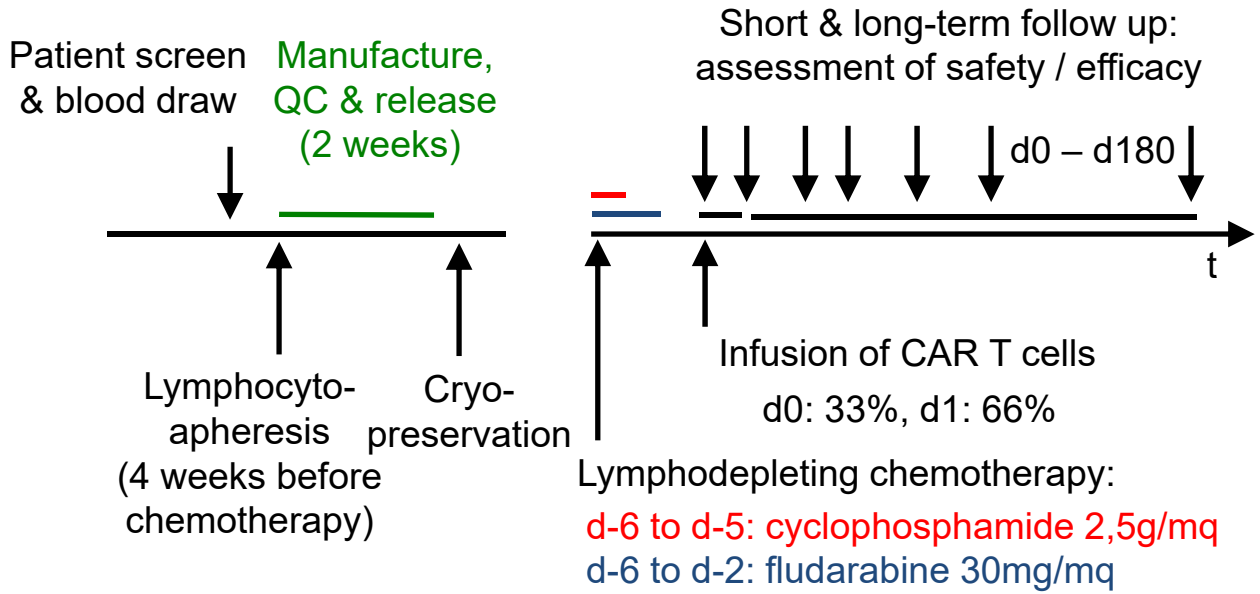


EURE CART

Participating centers:

MOLMED

San Raffaele, Milano (A Bondanza, C Bonini F Ciceri)
Wurzburg University (H Einsele)
Ospedale Pediatrico Bambino Gesù, Roma (F Locatelli)
Sant Pau Hospital, Barcelona (J Sierra)
University Hospital Ostrava (R Hajek)



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INTRODUCTION

The present document contains information on how to complete the Cell Therapy MED-A data collection form.

It is preceded by the definition of Cell therapy and information on when a new registration should be submitted to the EBMT. For general information on how to register data please visit http://www.ebmt.org/Contents/Data-Management/Pages/Data-Management.aspx

For downloads of the Cell therapy MED-A form and manual please go to http://www.ebmt.org/Contents/Data-Management/Registrystructure/MED-ABdatacollectionforms/Pages/MED-AB-data-collection-forms.aspx

For information on submitting data directly to the EBMT Registry using ProMISe software please refer to:
http://www.ebmt.org/Contents/Data-Management/Datasubmission/Pages/Data-Submission.aspx

Updated manuals are available to download from the above link. We are grateful for any feedback as to its content (clarity of the definitions, omissions, insufficient background or excessive verbosity, etc.). Please send all comments to the EBMT Registry Office at registryhelpdesk@ebmt.org

CELL THERAPY REGISTRY

The Cell Therapy Registry (CTR) aims to collect data on stem cells, progenitors or mature cells, such as T-lymphocytes, unmanipulated, such as DLI, or sorted and/or cultured and/or genetically manipulated, such as CAR-T cells, and including advanced therapeutic medicinal products (ATMP), used for treatment other than hematopoietic stem cell transplantation (HSCT) as well as data on the clinical characteristics and outcome of the treated patients.

The cells can be infused in combination with other treatments, including hematopoietic stem cell transplantation, or by themselves.

CETHORIG **Cell origin*:***

Indicate whether the cells infused proceeded from the patient or from another person.

Autologous the patient receives his/her own cells back

Allogeneic the patient receives a cellular therapy product prepared from cells harvested from another person

Product manufactured from

- a new donor –related or from a Donor registry- which has not been registered before but for which you have data.
- a donor that has been used before either for previous cell therapy or for a previous HSCT, and that has already been registered. If that is the case, you can select this option and there is no need to fill in the **Donor** section again.
- an unknown donor for whom there is no data. This may happen in some commercially manufactured products. If you select this option, the questions on the donor section can be skipped.

DONOR

DONRL **HLA match type**

Differences in histocompatibility (or degree of match) affect the outcome of cell infusions. We define histocompatibility by looking at differences in certain proteins (or antigens) between the patient and their donor (also called “tissue typing”). The antigens which are most important in the matching procedure are the major HLA-antigens. The genes encoding these antigens are found on chromosome 6. Each individual has two copies of chromosome 6, each copy inherited from one of the parents.

MANIGENE

Gene manipulation

Gene transfer

This is a procedure by which techniques of gene transfer/transduction are used to alter the structure and characteristics of genes in the graft before the cell infusion. This is an experimental procedure which is used in cases of inborn errors or cancer, but some products have been recently approved in Europe, and we may expect their extended use in the near future.

Retroviral vector – These vectors are used to genetically modify the cells. Add name of the used vector
Retroviruses are any group of RNA viruses that insert a DNA copy of their genome into the host cell to replicate. HIV is an example of a Retrovirus

Lentiviral vector - These vectors are used to genetically modify the cells. Add name of the used vector.
Lentiviruses are members of the genus of retroviruses that have long incubation periods and cause chronic, progressive, usually fatal disease in humans and other animals.

Other - specify the type of vector used. Non integrating vectors, including RNA electroporation, should be listed here.

N. of gene transfer cycles - Genetic manipulation can be performed by exposing cells to the vector once or more than once, to increase the efficiency of the procedure. List here the number of gene transfer cycles that were used to manufacture the cellular product infused to the patient.

Transgene - Transgenes are the genes that are introduced in the vector, and through the vector to the cells, to manufacture the cellular products.

Indicate the transgene used.

CAR	- specify the target recognized by the CAR
Suicide gene	- specify the name of the suicide gene
TCR	- specify the target recognized by the TCR and the HLA restriction element
Other	- specify the type and name of the transgene

Gene editing

Indicate if the cells underwent a type of genetic engineering in which DNA is inserted or removed from a genome using artificially engineered nucleases. Artificially engineered nucleases include Zinc Finger Nucleases (ZFN), TALEN and CRISPRR/Cas9. Specify the gene manipulated.

Recognition of a specific target / antigen.
In some cases the cellular products are specific for a target: ie: CART cells (specific for CD19, or other molecules) or viral specific T cells. If this is the case, please specify the target.

Selection

CTIUSELECT **Positive**
Selection of cells for example by the monoclonal antibody that select only certain types of lymphocytes. The selected cells are used for the cell therapy.

Negative
Cells are destroyed (or removed) from the product, either with some drug like cyclophosphamide derivatives, or with specific antibodies that bind to them.

Purity: This is the percentage of the selected cells among the total number of cells in the final product. For example, if T cells are to be infused and after the manipulation the end cellular product is composed of 99% T cells and 1% NK cells, purity will be 99%.

Yield: This is the percentage of selected cells in the final product against the total number of those same cells in the pre- manipulated product. For example, if the unmanipulated product contained 50 T cells, and after the selection process, only 40 T cells are left, the yield is 40/50x100=80%

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AIRC


5x1000


TRANSCAN


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PER IL PROGRESSO DELLE SCIENZE


REPVBBLICA ITALIANA
Ministero della Salute


EBMT


European
Commission


RegioneLombardia


OSPEDALE SAN RAFFAELE


ADIENNE
PHARMA & BIOTECH


ITALFARMACO


MOLMED


Cell
Teaching your cells to treat your disease