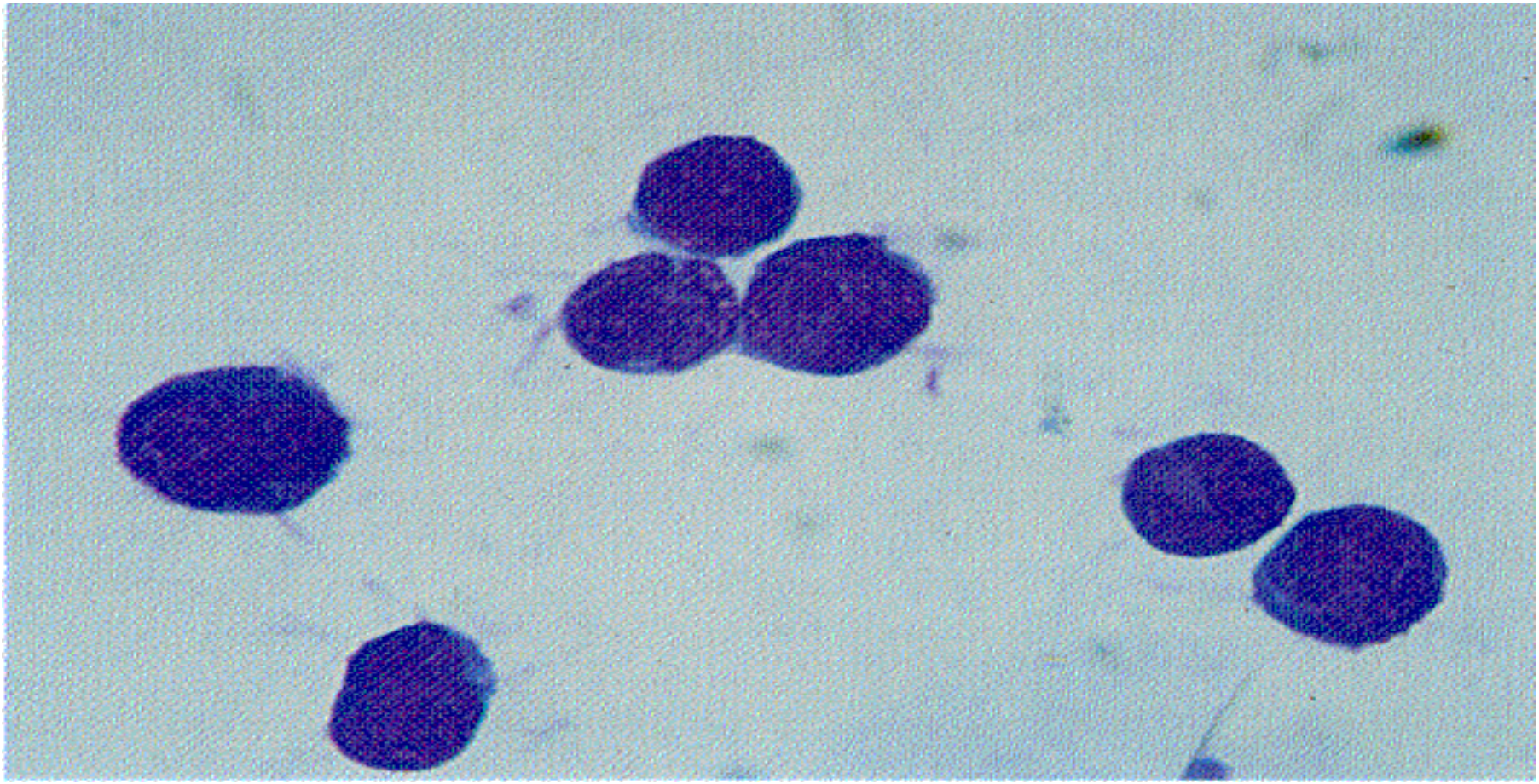
RACCOLTA E TRAPIANTO DI CELLULE STAMINALI EMOPOIETICHE

TRAPIANTO DI CELLULE STAMINALI EMOPOIETICHE

E' la trasfusione di cellule staminali emopoietiche prelevate in quantita' adeguata da un donatore idoneo (*trapianto allogenico*) o dal paziente stesso (*trapianto autologo*)

HEMATOPOIETIC STEM CELL TRANSPLANTATION

TYPE OF TRANSPLANT	*DONOR*	*HLA MATCHING*	*SOURCE OF HSC*
SYNGENIC	SIBLING	IDENTICAL	BM or PB
ALLOGENIC	SIBLING UNRELATED SIBLING PARENT CHILD	IDENTICAL IDENTICAL or MISMATCHED MISMATCHED	BM, PB, CB
AUTOLOGOUS	SELF		BM or PB



FREQUENZA DELLE CELLULE STAMINALI CD34

- ***MIDOLLO OSSEO:***
  - **CONDIZIONI BASALI** **1%**
  - **STIMOLATO** **5%**
- ***SANGUE Pperiferico***
  - **CONDIZIONI BASALI** **0.1%**
  - **STIMOLATO** **1%**

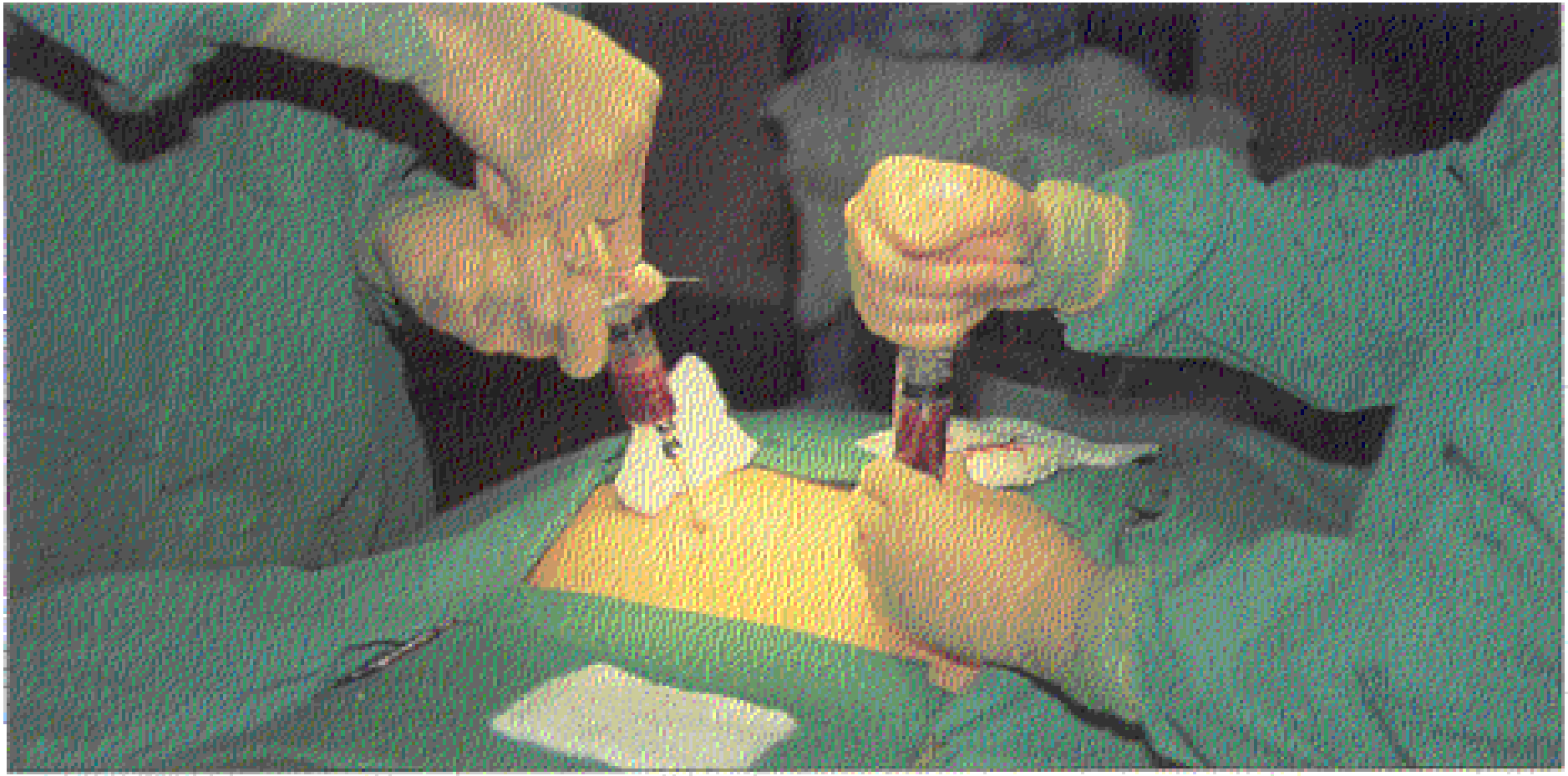
RACCOLTA CELLULE STAMINALI
MIDOLLO

ASPIRAZIONI MULTIPLE DALLA CRESTA ILIACA POSTERIORE IN ANESTESIA GENERALE.

(cresta iliaca anteriore e sterno)

IL MIDOLLO VIENE ASPIRATO, PROCESSATO E QUINDI CRIOPRESERVATO O SUBITO REINFUSO

RACCOLTA DI CELLULE STAMINALI DA MIDOLLO OSSEO

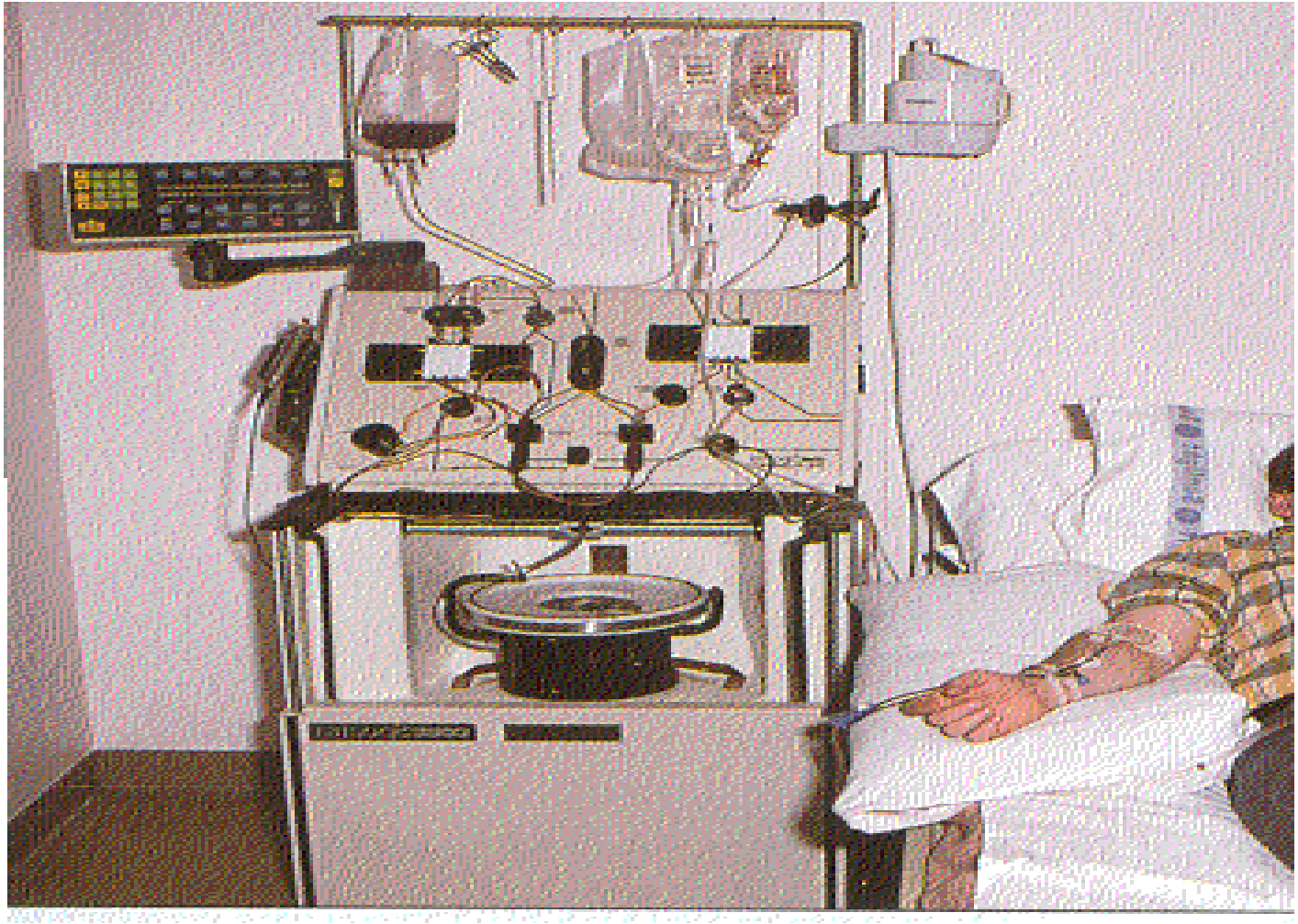


RACCOLTA CELLULE STAMINALI
SANGUE PERIFERICO

- La raccolta di cellule staminali da sangue periferico avviene tramite passaggio del sangue in una macchina che e' in grado di identificare le cellule staminali e di trattenerle, restituendo tutte le altre cellule al paziente.

- Le cellule staminali cosi' raccolte vengono concentrate e criopreservate o subito reinfuse.

RACCOLTA DI CELLULE STAMINALI DA SANGUE PERIFERICO



Criopreservazione delle cellule staminali emopoietiche (CSE)

- CSE vitali per 2-3 giorni a temperature > a t. di congelamento
- La criopreservazione mantiene inalterate le caratteristiche biologiche oltre i limiti di tempo naturali.
- Si esegue con congelatore programmato in azoto liquido

- **Possibili danni della crioconservazione**:

1. Cristalli di ghiaccio e danno degli organuli cellulari
2. Cristalli di ghiaccio extracellulari e disidratazione delle cellule
3. Reidratazione ed espansione delle cellule durante lo scongelamento
4. Funzionamento parziale degli enzimi cellulari a basse temperature con possibile accumulo di metaboliti tossici

Criopreservazione delle cellule staminali emopoietiche (CSE)

Crioconservante:
dimetilsulfossido (DMSO) al 5-10%
(disidratazione parziale intracellulare)

Principi base:

- DMSO aggiunto al plasma pochi ml alla volta mentre viene raffreddato su ghiaccio (reazione esotermica) → midollo

- Viene mantenuto lo stesso profilo termico delle componenti della miscela

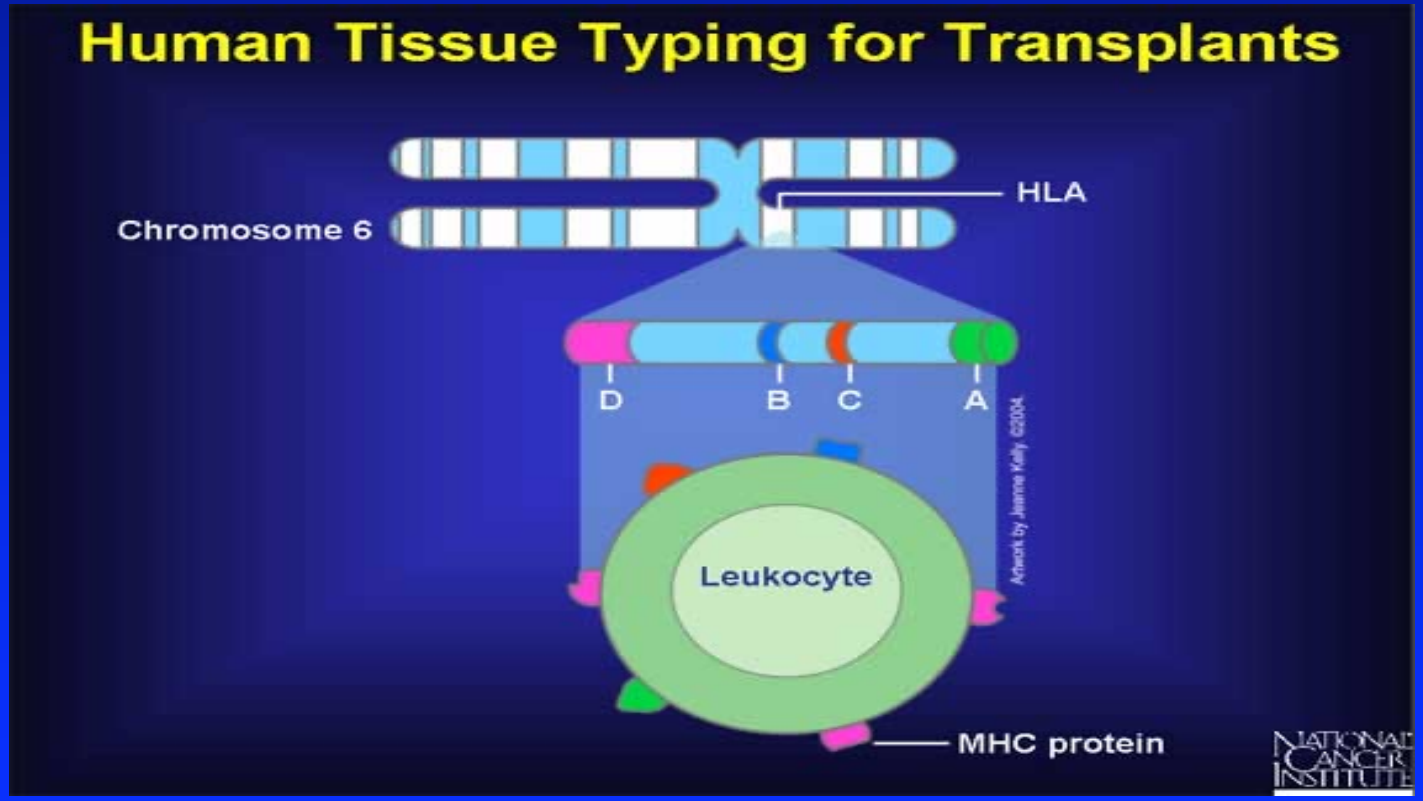
Crioconservazione e scongelamento

- A lungo termine a -196 °C, a -80°C per brevi periodi
- Tempo 3-5 anni (max 10 anni ma con controllo della vitalità)
- Scongelamento delle sacche a bagno maria (37-38°C) nel più breve tempo possibile
- Reinfusione subito dopo lo scongelamento

Trapianto di cellule staminali emopoietiche autologhe

- Condizionamento: intensificazione della chemioterapia ovvero chemioterapia ad alte dosi
- Reinfusione delle CSE autologhe: funzione di “salvataggio”
- Efficacia terapeutica dal regime di condizionamento NON dalla reinfusione delle CSE autologhe

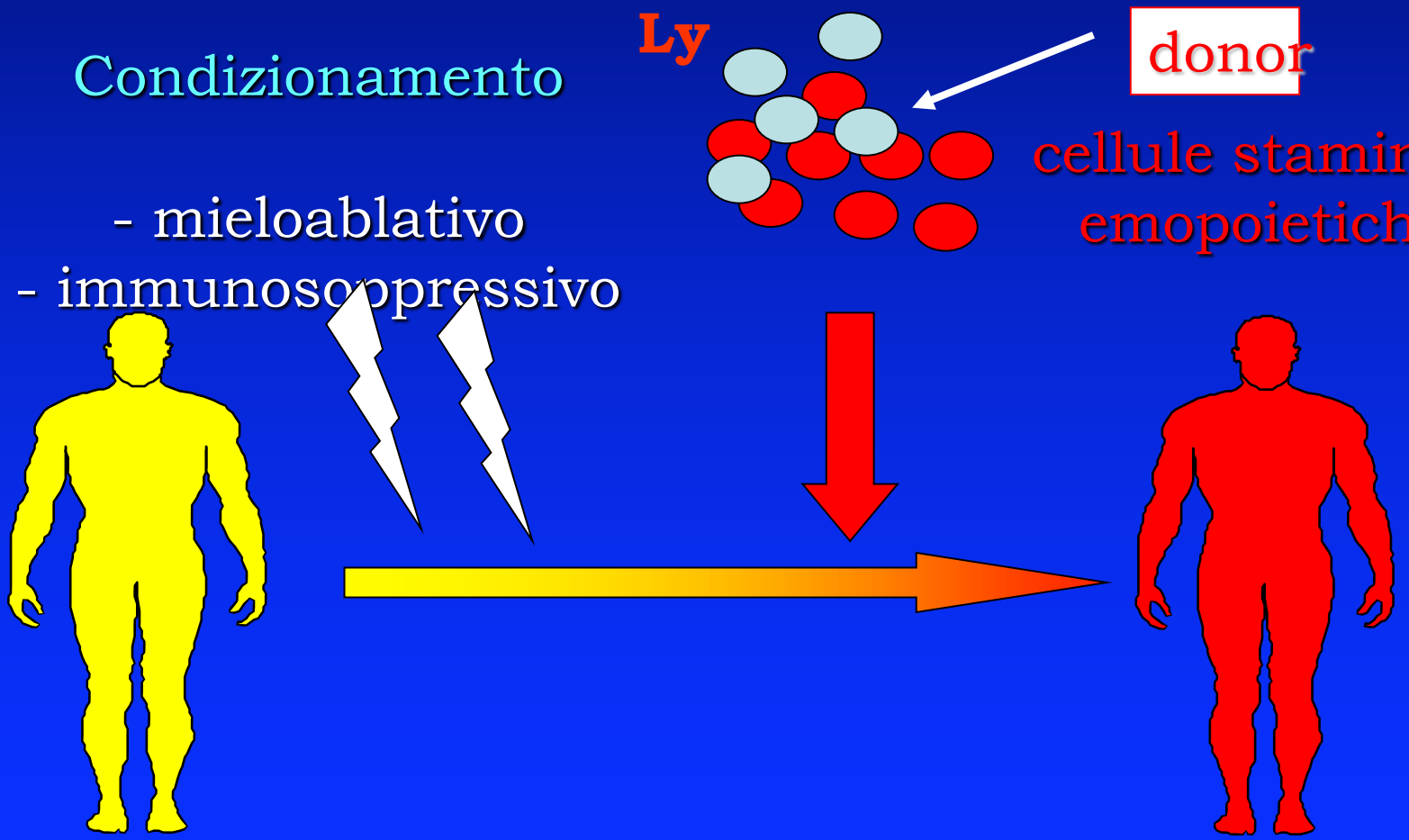
Trapianto di CSE allogeniche e sistema HLA



Trapianto allogenico di cellule staminali emopoietiche

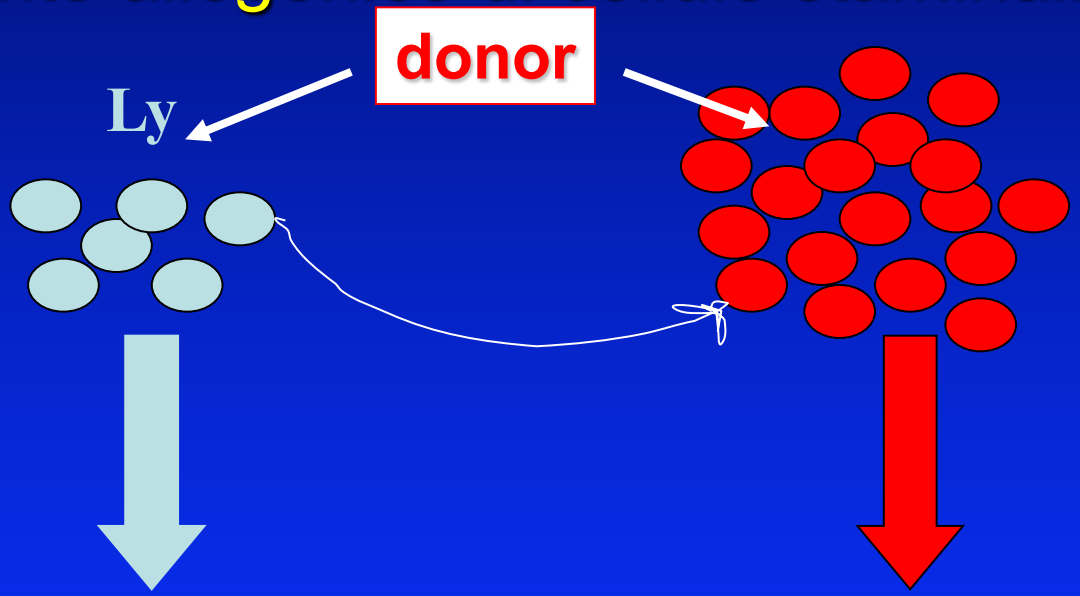
Condizionamento

- mieloablativo
- immunosoppressivo



chimerismo completo

Trapianto allogenico di cellule staminali emopoietiche



cellule staminali emopoietiche

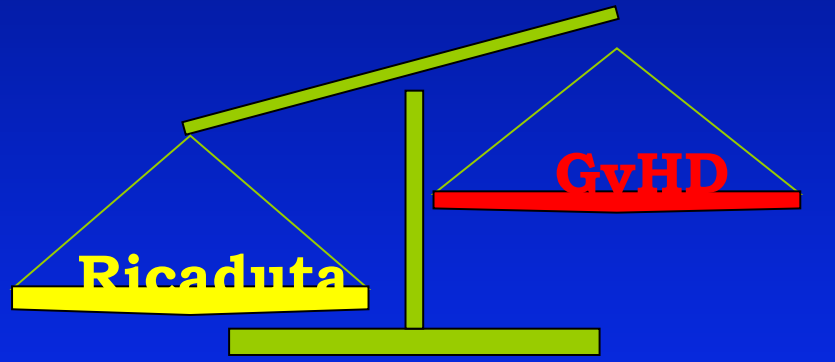
effetto anti-tumorale (GVL)

reazione del trapianto verso l'ospite (GVHD)

ricostituzione emopoietica

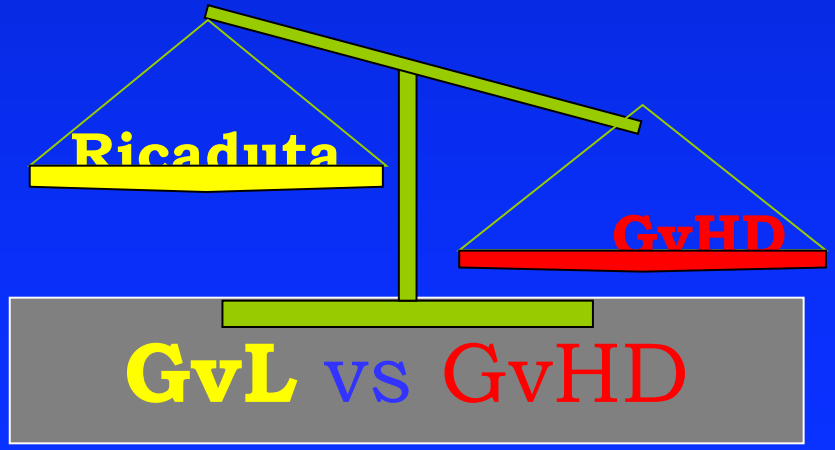
Transplant Related Mortality (TRM)

Graft-*versus*-Host reaction



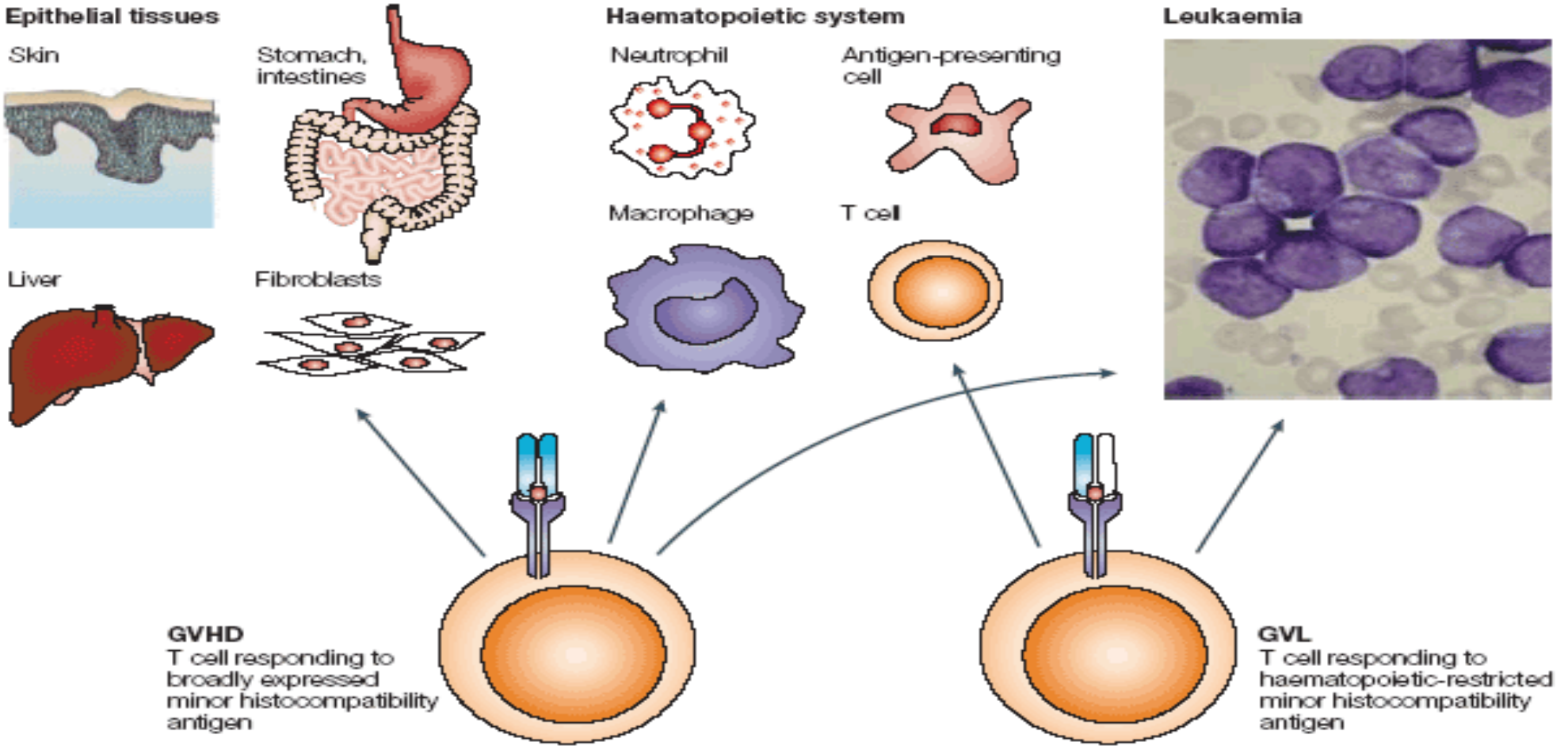
Minore GvHD - GVL

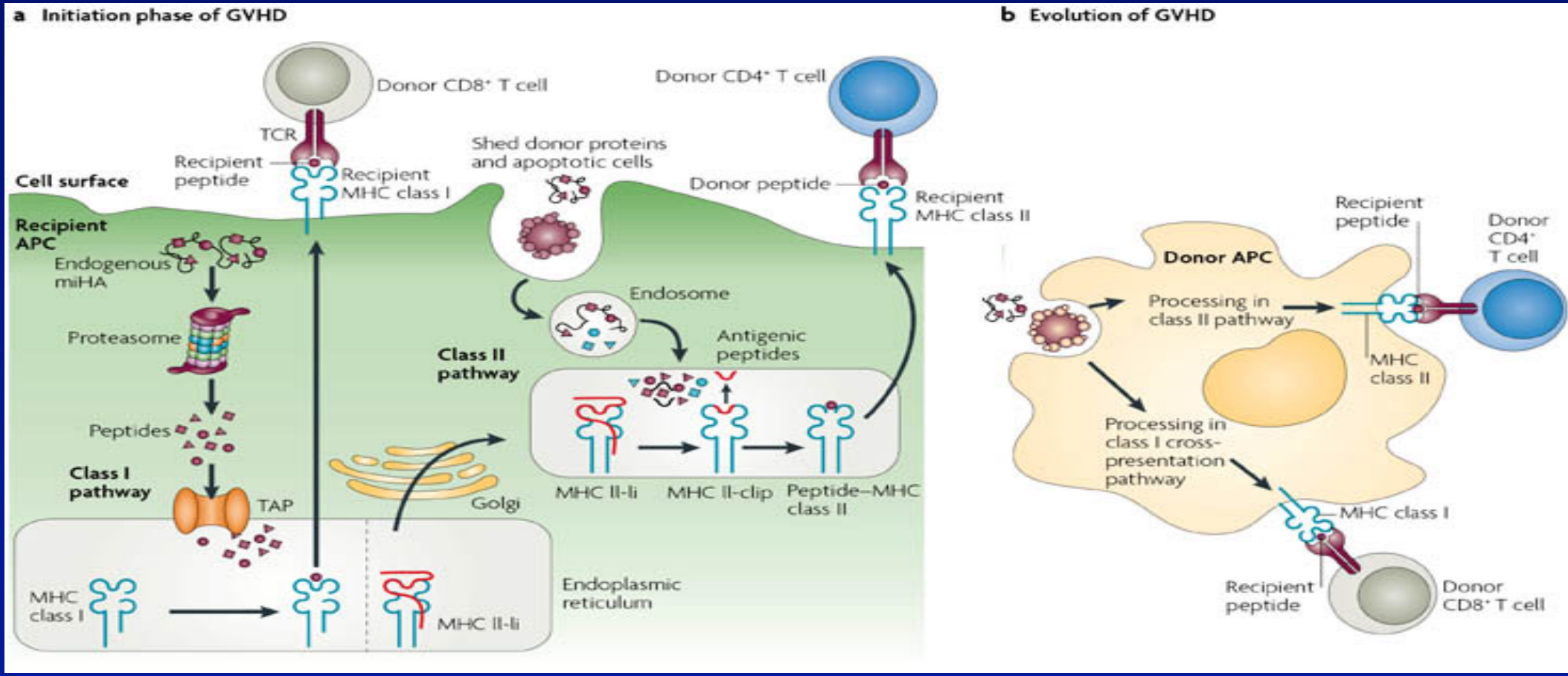
Maggiore prob. di ricaduta

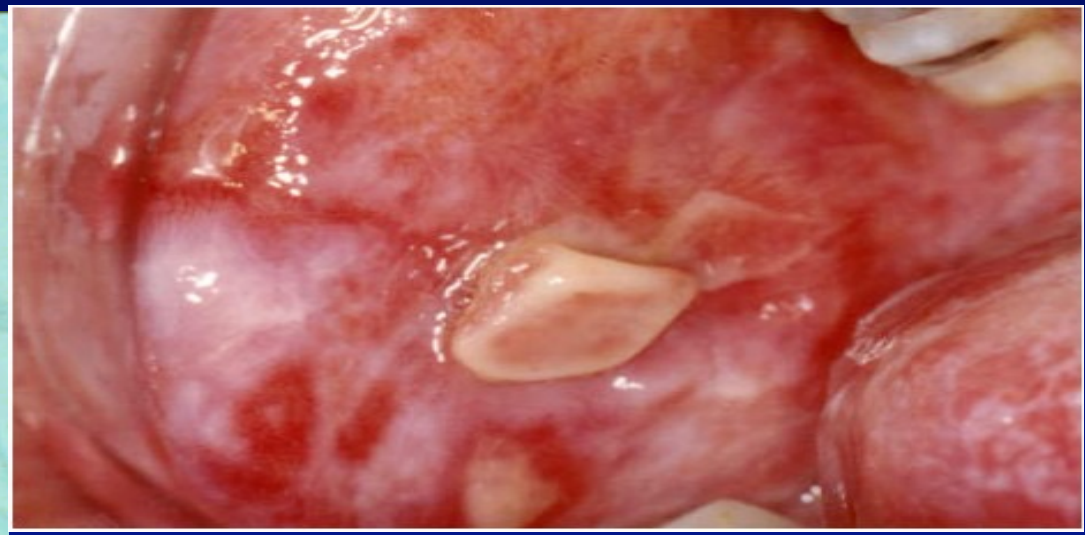


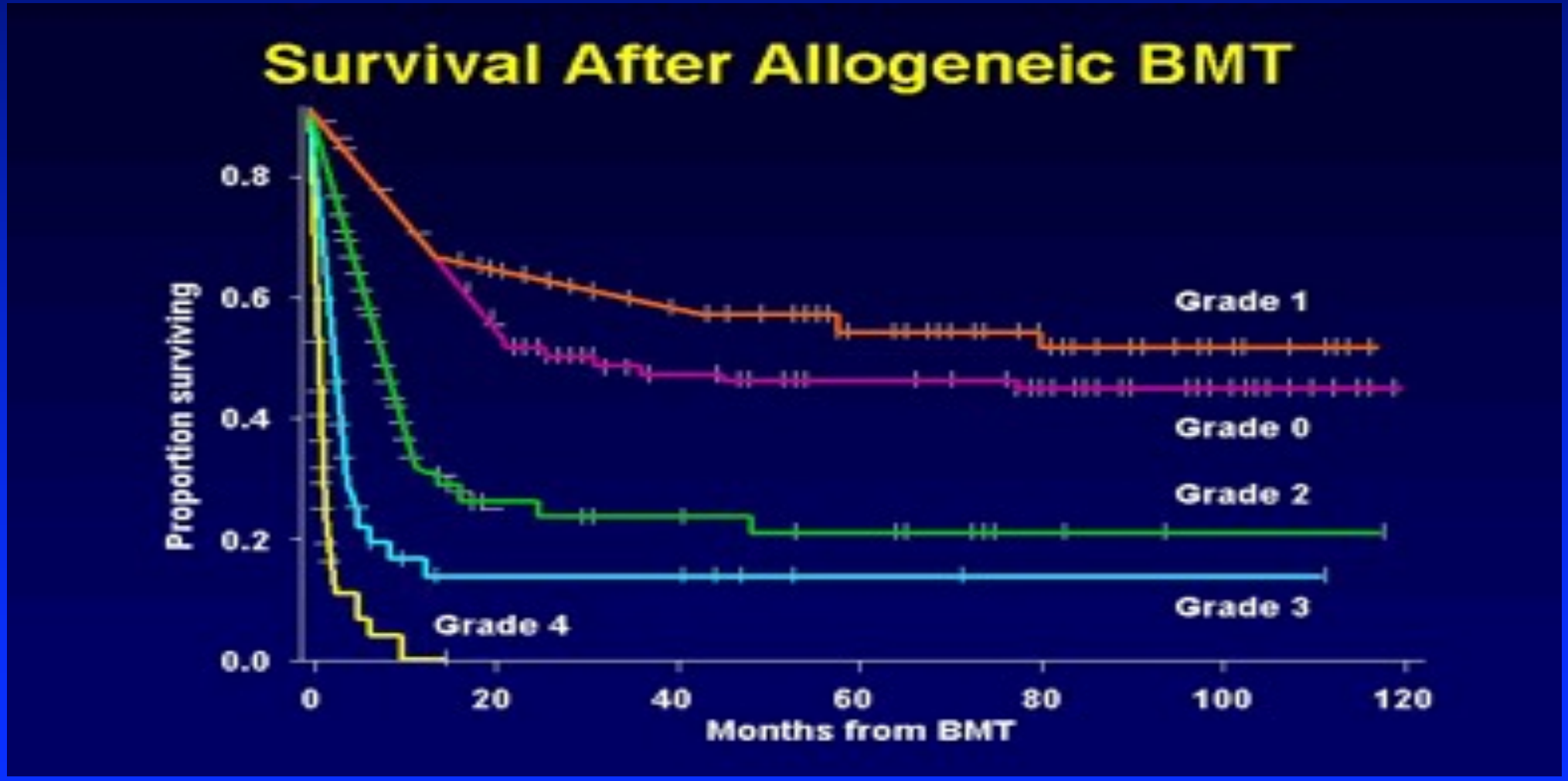
Maggiore GvHD + GVL

Minore prob. di ricaduta




a Initiation phase of GVHD
Donor CD8+ T cell
TCR
Recipient peptide
Recipient MHC class I
Cell surface
Recipient APC
Endogenous miHA
Proteasome
Peptides
Class I pathway
TAP
MHC class I
Shed donor proteins and apoptotic cells
Endosome
Class II pathway
Antigenic peptides
Golgi
MHC II-Ii
MHC II-clip
Peptide–MHC class II
Endoplasmic reticulum
MHC II-Ii
Donor CD4+ T cell
Donor peptide
Recipient MHC class II
b Evolution of GVHD
Donor APC
Recipient peptide
Donor CD4+ T cell
Processing in class II pathway
MHC class II
Processing in class I cross-presentation pathway
MHC class I
Recipient peptide
Donor CD8+ T cell

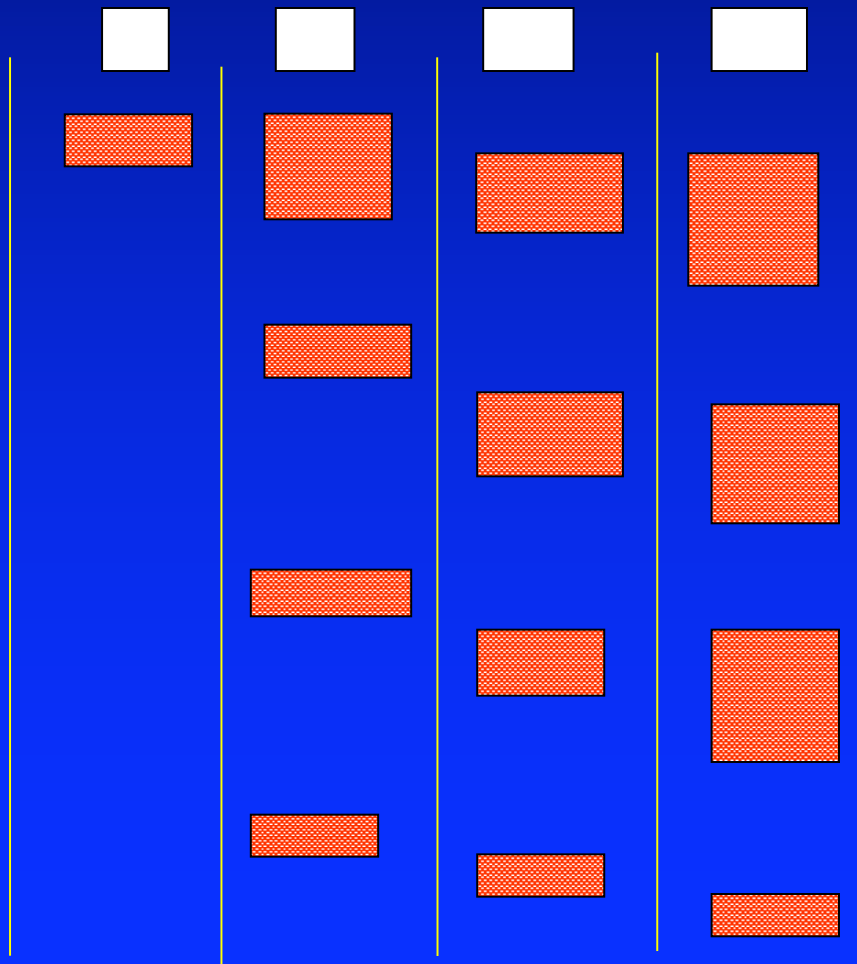



Survival After Allogeneic BMT
Proportion surviving
0.0
0.2
0.4
0.6
0.8
0
20
40
60
80
100
120
Months from BMT
Grade 1
Grade 0
Grade 2
Grade 3
Grade 4

GRADING GVHD

ORGANO	ESTENSIONE		STADIO
CUTE	RASH < 25%		+
	25-50%		++
	> 50%		+++
	BOLLE		++++
FEGATO		2-3	+
	BILIR.	3.1-6	++
	mg%	6.1-15	+++
		>15	++++
INTESTINO	DIARREA ml/die	>500	+
		>1.000	++
		>1.500	+++
	DOLORE ILEO		++++
PERF.ST.	COMPROMISS.		+
			++
			+++

GRADO



SIGNS & SYMPTOMS of Chronic GVHD

ORGAN/SITE	
SKIN	Erythema, maculopapular rash, pruritus, poikiloderma, lichen-like features
NAILS	dystrophy
SCALP	alopecia
MOUTH	Lichen, xerostomia, mucositis
EYES	Keratoconjunctivitis sicca, photophobia, blepharitis
GI tract	Strictures of oesophagus, anorexia, weight loss
LIVER	Bilirubin or ALP > 2 x ULN
LUNG	BOS
MUSCLES, JOINTS	Fascitis, joint contractures, myositis, cramps, arthralgias
HEMATOPOIETIC &	Thrombocytopenia, eosinophilia, lymphopenia

Un CONDIZIONAMENTO MIELOABLATIVO è la "*conditio sine qua non*" per eseguire un ALLO-SCT e curare una neoplasia ematologica?

- Maggiore RR dei pz sottoposti ad Auto-SCT/Singenico *vs* Allo-SCT
- ↑ RR dopo T-deplezione
- ↓ RR in pz che sviluppano aGVHD e/o cGVHD
- RC post DLI

Un CONDIZIONAMENTO MIELOABLATIVO è la "*conditio sine qua non*" per eseguire un ALLO-SCT e curare una neoplasia ematologica?

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Esiste una GVT, mediata dai Ly T del donatore, diretta verso Ag minori dell'HLA e Ag associati alla neoplasia

Un CONDIZIONAMENTO MIELOABLATIVO è la "*conditio sine qua non*" per eseguire un ALLO-SCT e curare una neoplasia ematologica?

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- ↓ RR in pz che sviluppano aGVHD e/o cGVHD
- RC post DLI

↓

Esiste una GVT, mediata dai Ly T del donatore, diretta verso Ag minori dell'HLA e Ag associati alla neoplasia

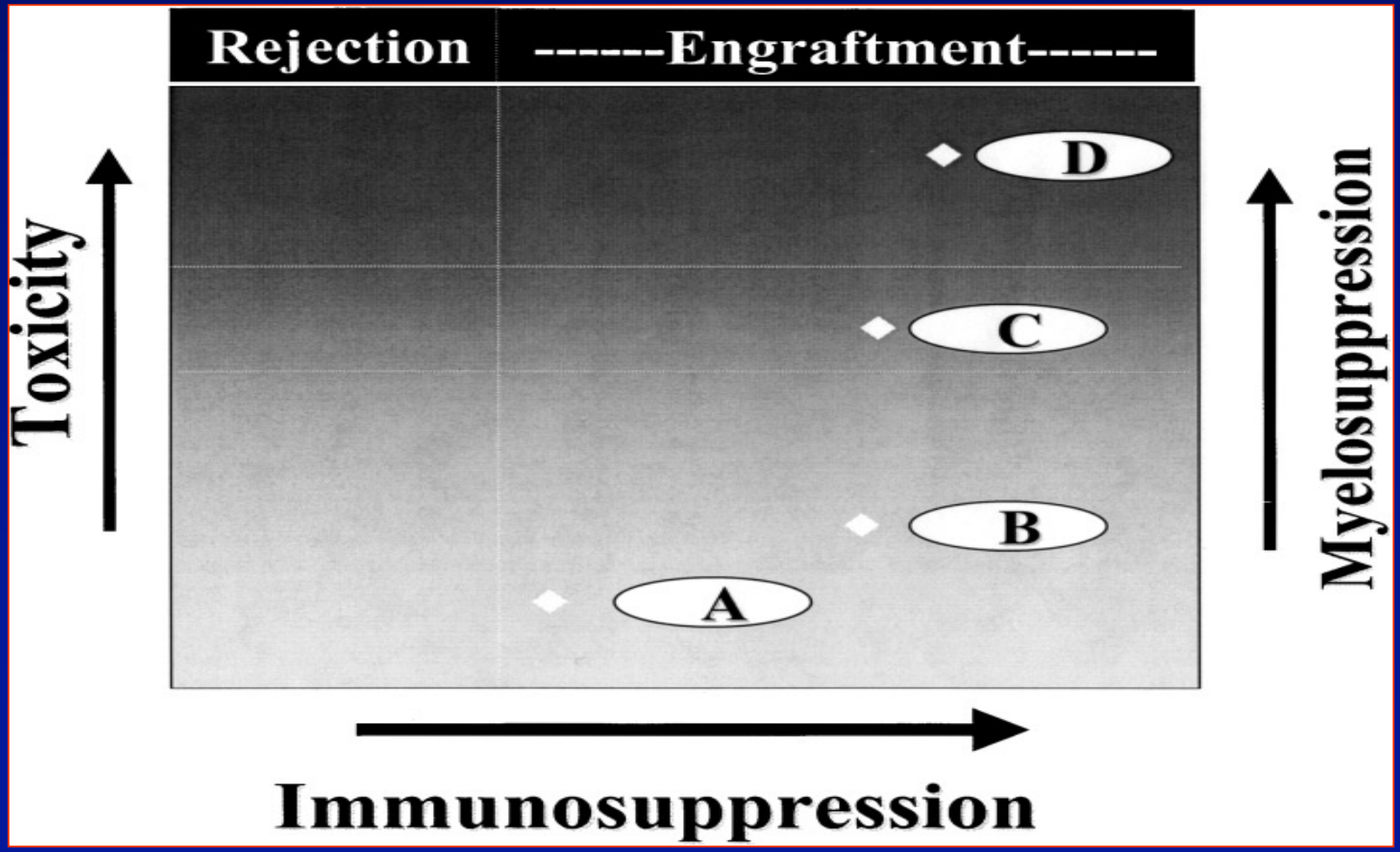
↓

- Condizionamento mieloablativo (>Tox)
- Condizionamento ad intensità ridotta (< Tox)
- Condizionamento immunosoppressivo (<< Tox)

Su che cosa si basa un TRAPIANTO DI CSE NON MIELOABLATIVO ?

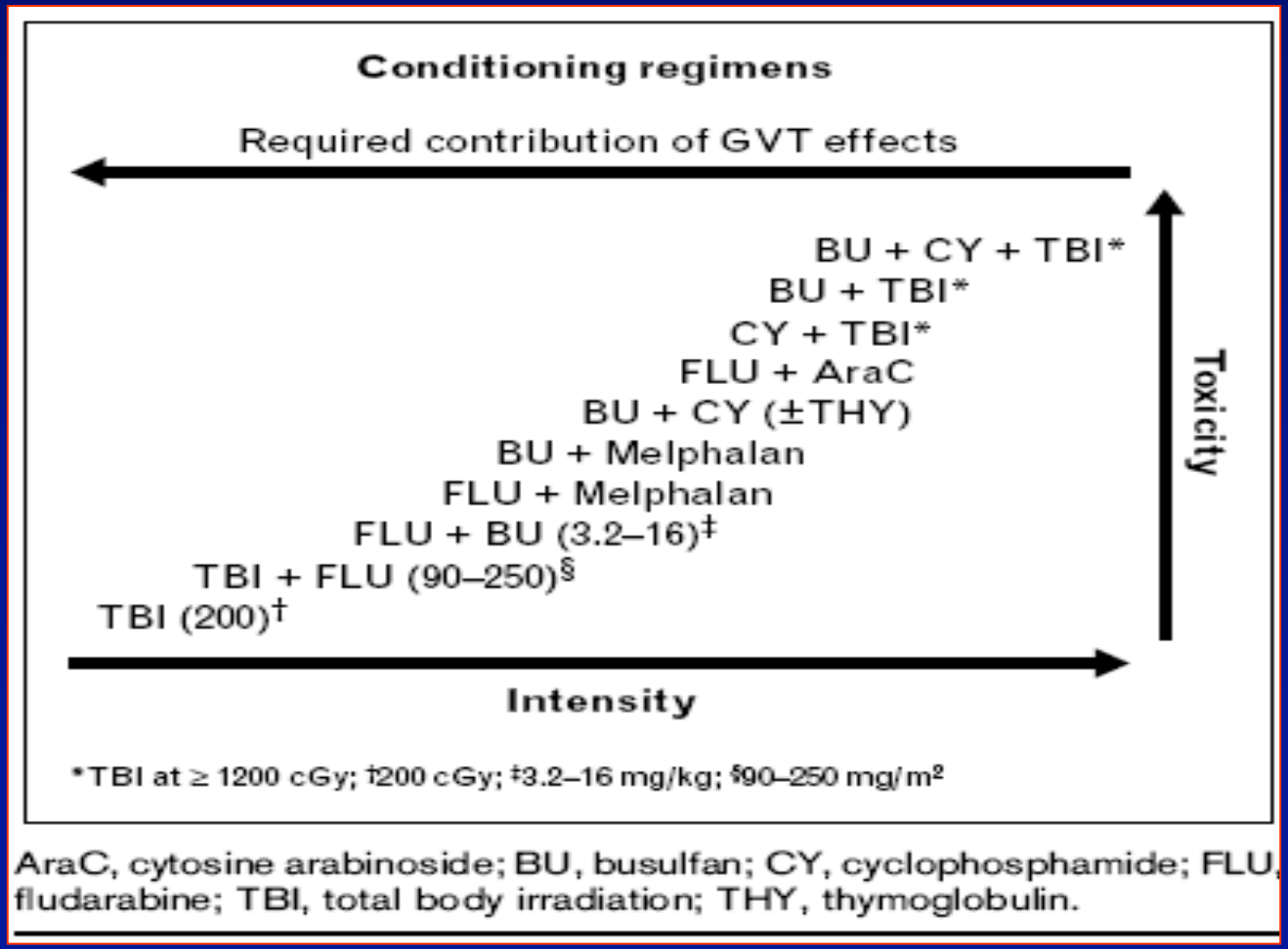
- CONDIZIONAMENTO AD INTENSITA' RIDOTTA
- CHIMERISMO MISTO DONATORE/RICEVENTE
- GRAFT *versus* TUMOR PIU' VELOCE E COMPLETA

Esiste un solo tipo di Allo-SCT non mieloablativo?



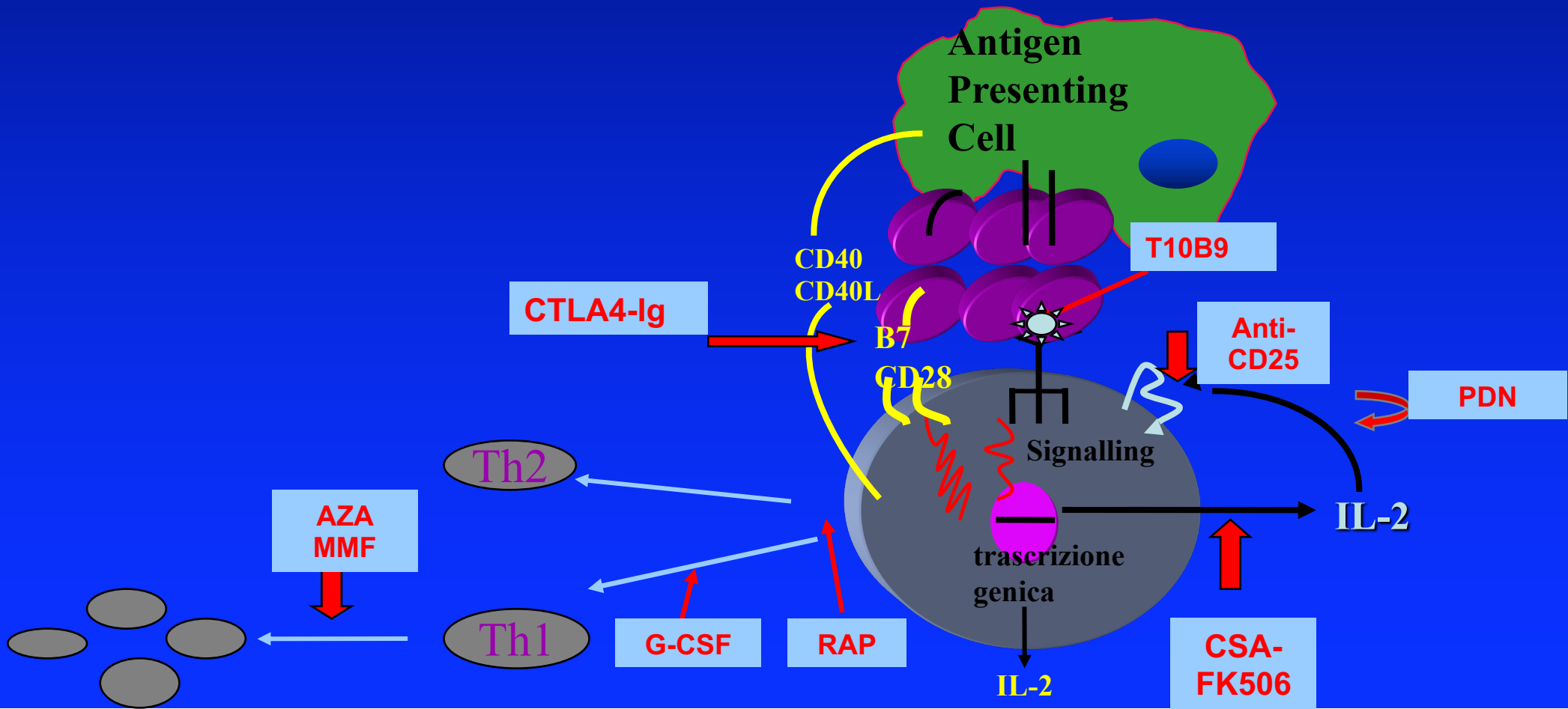
Barrett et al, BJH 2000

Esiste un solo tipo di Allo-SCT non mieloablativo?



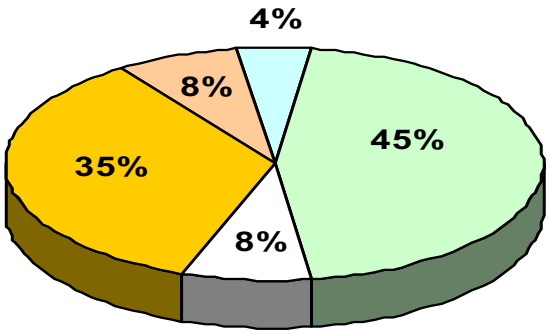
AraC, cytosine arabinoside; BU, busulfan; CY, cyclophosphamide; FLU, fludarabine; TBI, total body irradiation; THY, thymoglobulin.

GVHD

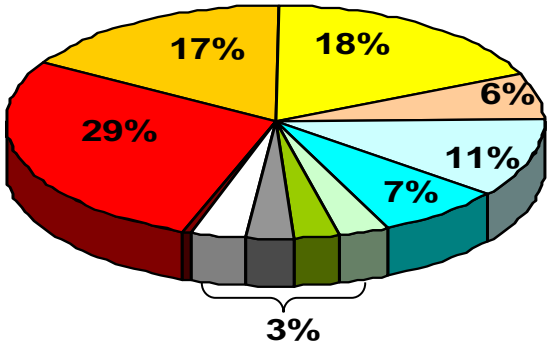

Antigen Presenting Cell
T10B9
CD40
CD40L
CTLA4-Ig
B7
CD28
Anti-CD25
PDN
Signalling
IL-2
trascrizione genica
Th2
AZA
MMF
Th1
G-CSF
RAP
IL-2
CSA-FK506

Patologie dei pazienti in ricerca

Patologie dei pazienti in ricerca nel 1990



Patologie dei pazienti in ricerca nel 2009

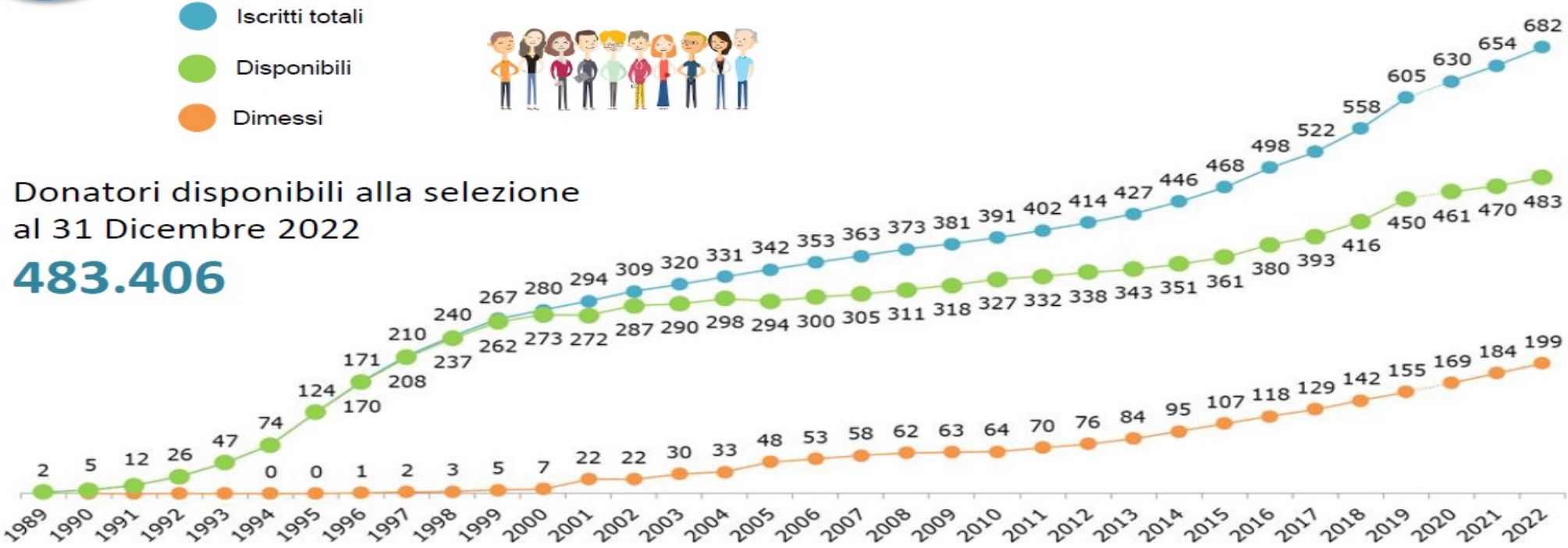


- Leucemia mieloide acuta
- Leucemia linfoblastica acuta
- Linfomi
- Mielodisplasia alto rischio
- Immunodeficienze e disordini congeniti
- Mieloma multiplo
- Leucemia mieloide cronica
- Talassemia
- Mielofibrosi idiopatica
- Anemia aplastica severa
- Neuroblastoma

Trend dell'inventario IBMDR

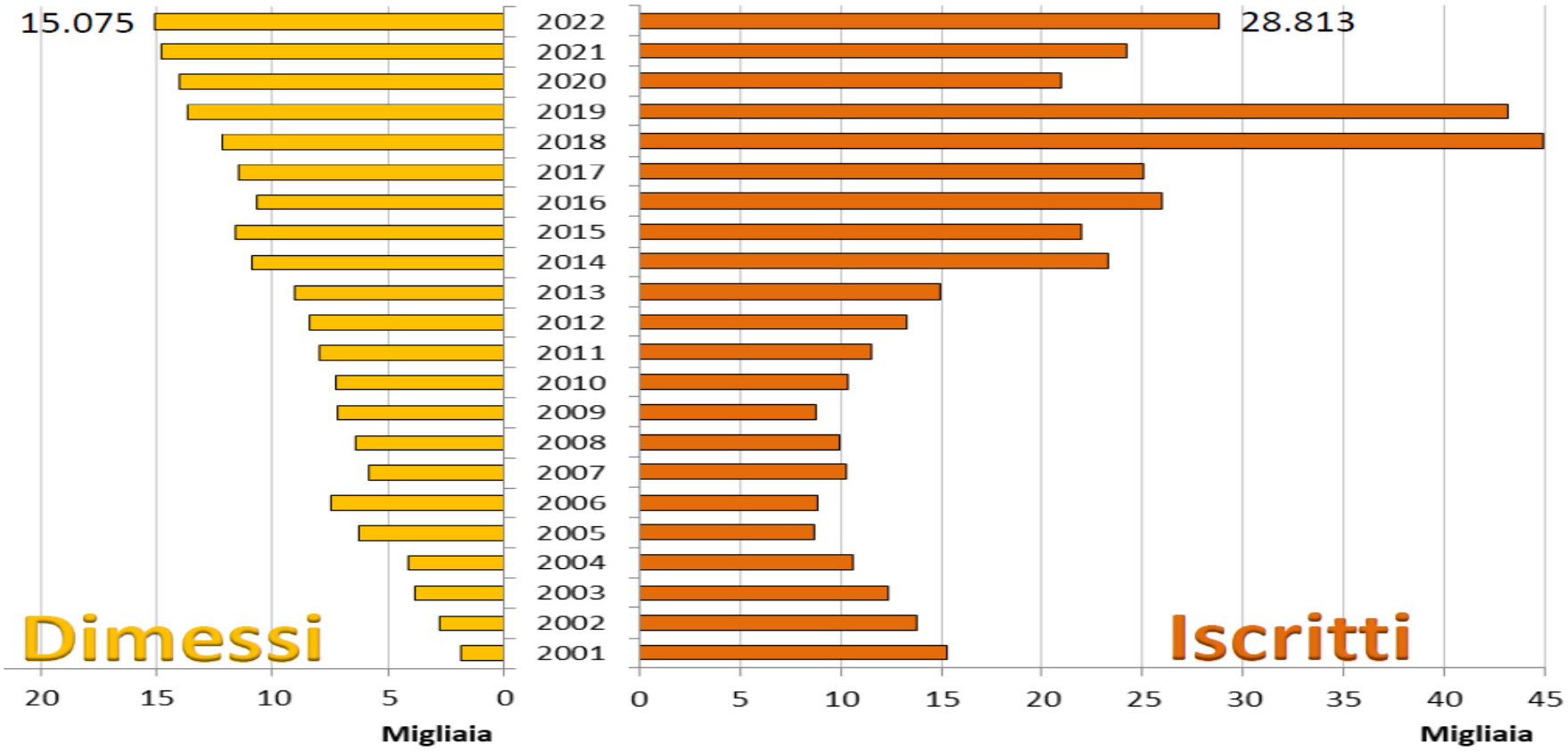
Donatori disponibili alla selezione
al 31 Dicembre 2022

483.406



* Gli iscritti totali comprendono tutti i donatori, anche quelli non più rispondenti ai requisiti di reclutamento

Donatori reclutati e dimessi per anno al 31 Dicembre 2022



Bilancio IBMDR - donatori reclutati

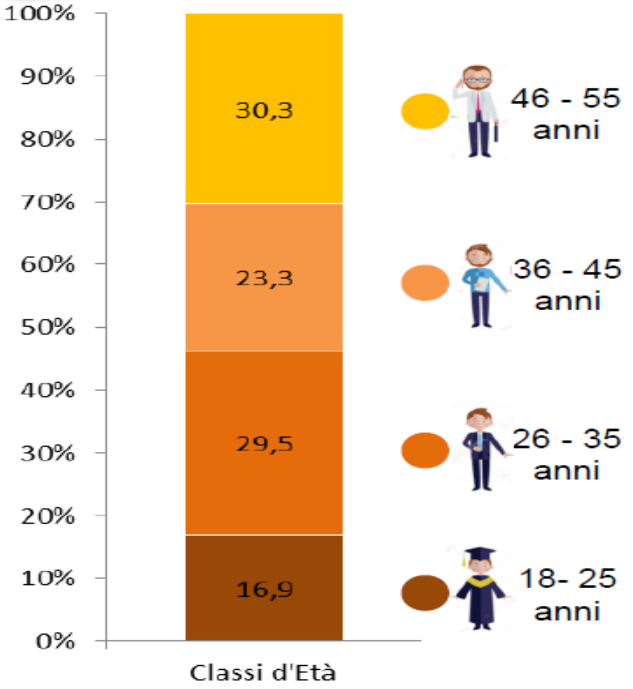
REGIONE	INSERITI IN RICERCA	*DIMESSI	BILANCIO
ABRUZZO E MOLISE	265	199	66
BASILICATA	58	130	-72
CALABRIA	166	256	-90
CAMPANIA	675	126	549
EMILIA ROMAGNA	4888	1862	3026
FRIULI VG	1066	399	667
LAZIO	2237	529	1708
LIGURIA	310	596	-286
LOMBARDIA	5175	3420	1755
MARCHE	403	366	37
PIEMONTE	3399	1491	1908
Prov. BZ	420	193	227
Prov. TN	882	180	702
PUGLIA	1665	463	1202
SARDEGNA	726	868	-142
SICILIA	501	358	143
TOSCANA	909	952	-43
UMBRIA	63	169	-106
VALLE D'AOSTA	45	31	14
VENETO	4978	2158	2820
TOTALE	28831	14746	14085

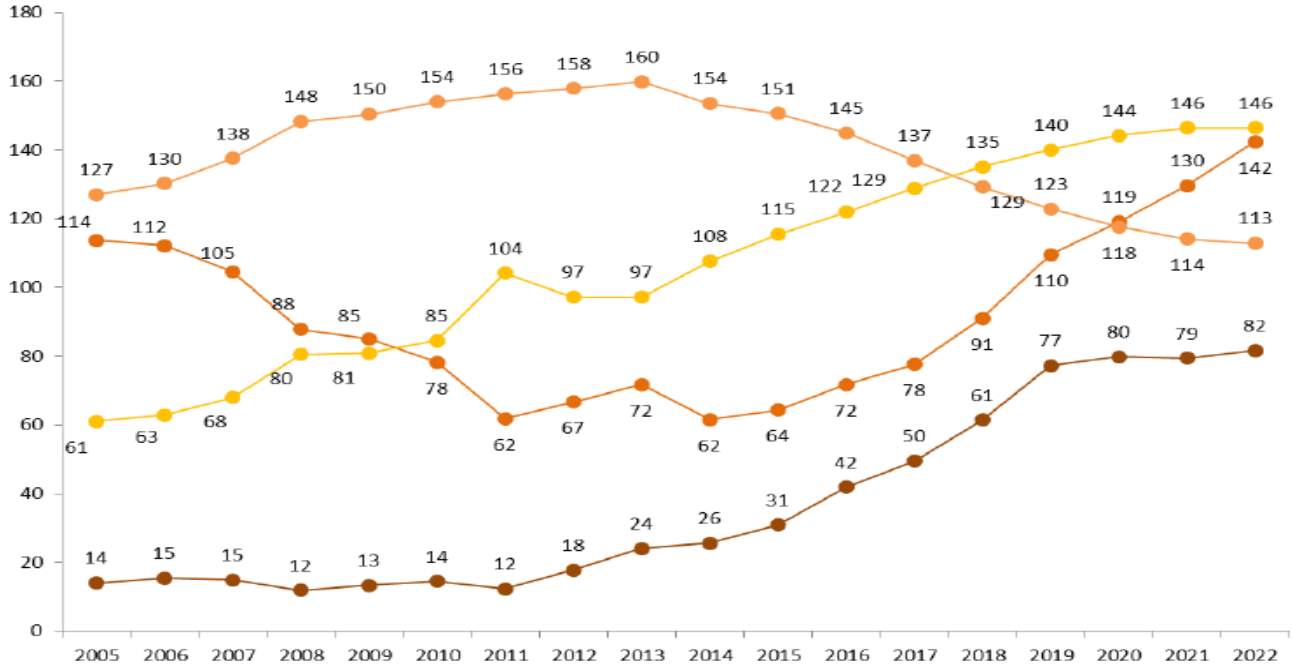
Bilancio potenziali nuovi donatori reclutati e dimessi per regione al 31 Dicembre 2022

+3%

*Sono esclusi i dimessi per donazione

Età dei potenziali donatori IBMDR





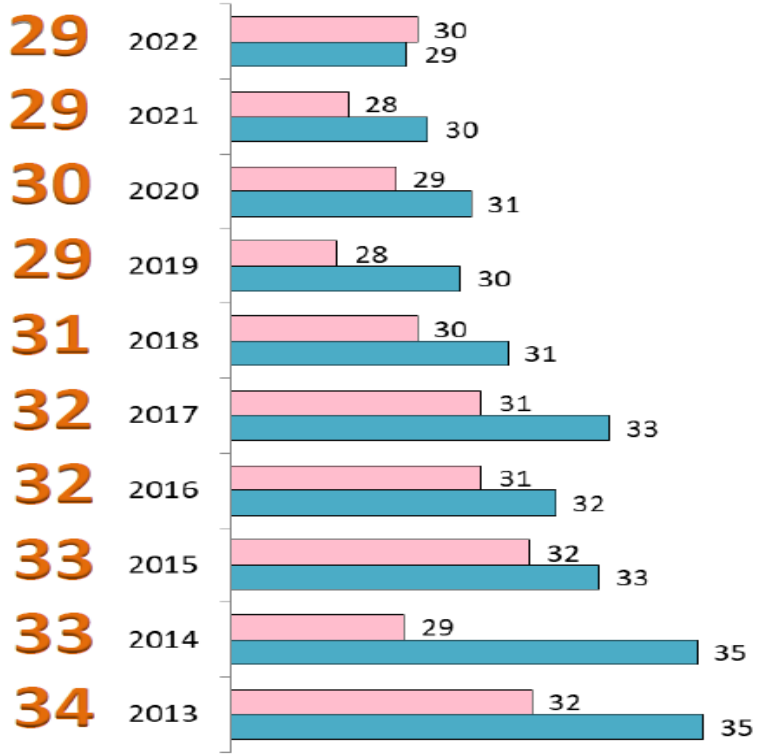
DONATORI DISPONIBILI ALLA DONAZIONE

Andamento della distribuzione per **classi d'età***

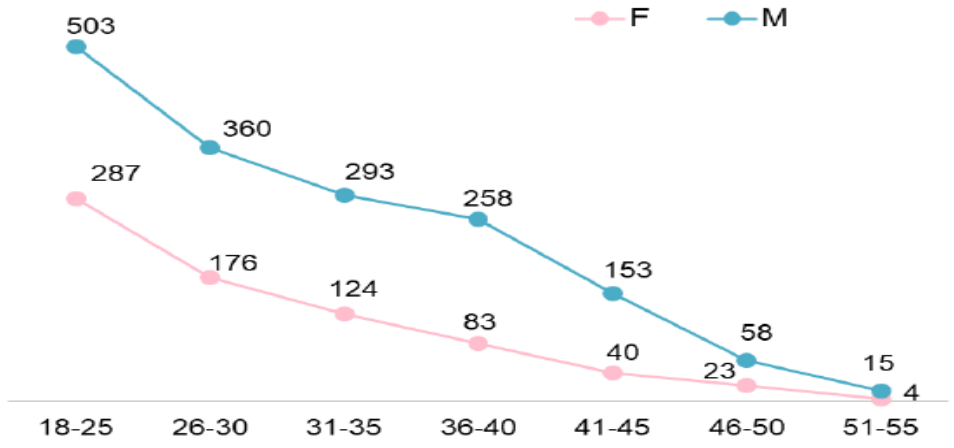
* Età dei donatori calcolata al 31 Dicembre

Caratteristiche donatori effettivi IBMDR dal 2013 al 2022

Età media alla donazione

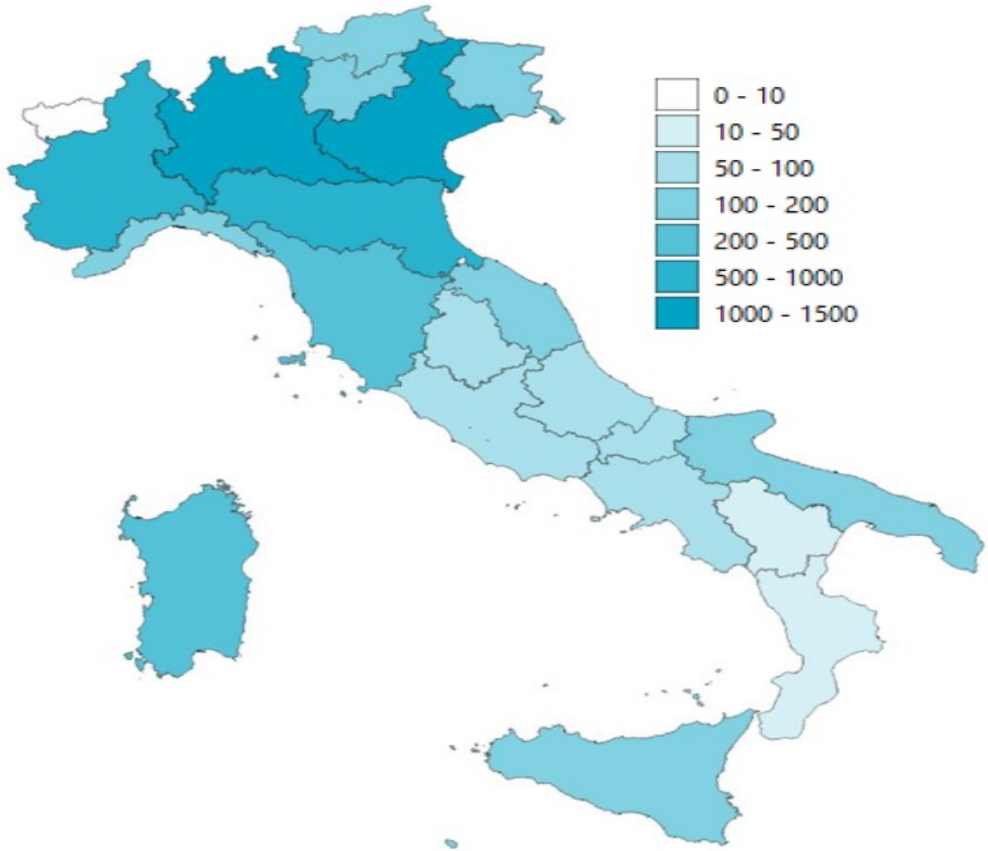


Rapporto maschi/femmine selezionati per donazione

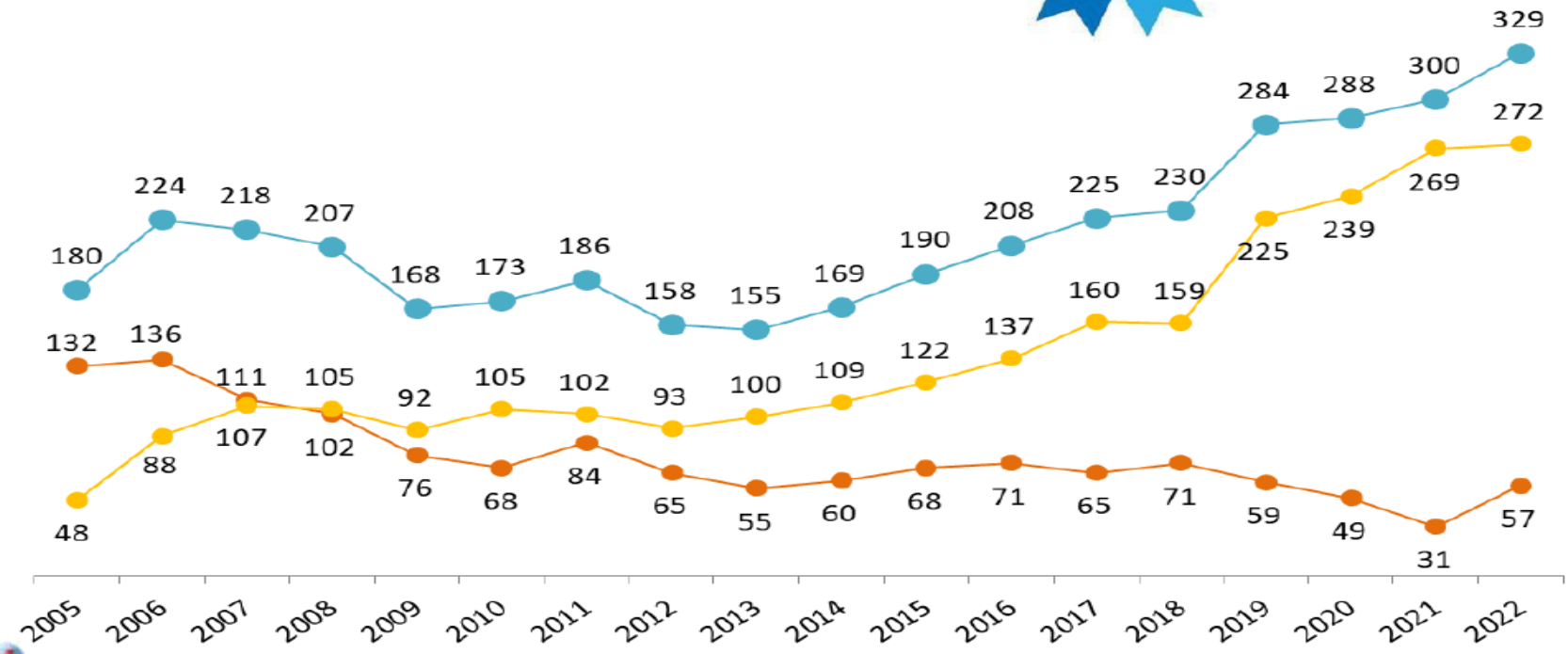


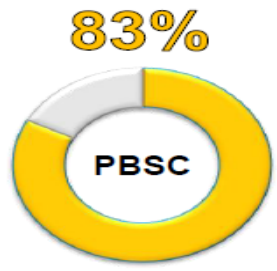
Numero totale prime donazioni IBMDR e sorgente CSE

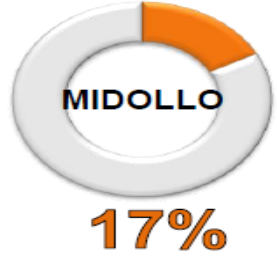
REGIONE	Prime donazioni Midollo	Prime donazioni PBSC	Totale
Abruzzo-Molise	35	30	65
Basilicata	15	13	28
Calabria	20	13	33
Campania	25	27	52
CSR - Sanit. Esercito	15	6	21
Emilia-Romagna	232	269	501
Friuli-Venezia Giulia	87	82	169
Lazio	48	28	76
Liguria	102	36	138
Lombardia	698	527	1225
Marche	55	49	104
Piemonte	268	350	618
Prov. autonoma BZ	56	52	108
Prov. autonoma TN	35	67	102
Puglia	102	83	185
Sardegna	158	127	285
Sicilia	54	66	120
Toscana	94	113	207
Umbria	52	2	54
Valle d'Aosta	2	4	6
Veneto	538	587	1125
ITALIA	**2691**	**2531**	**5222**



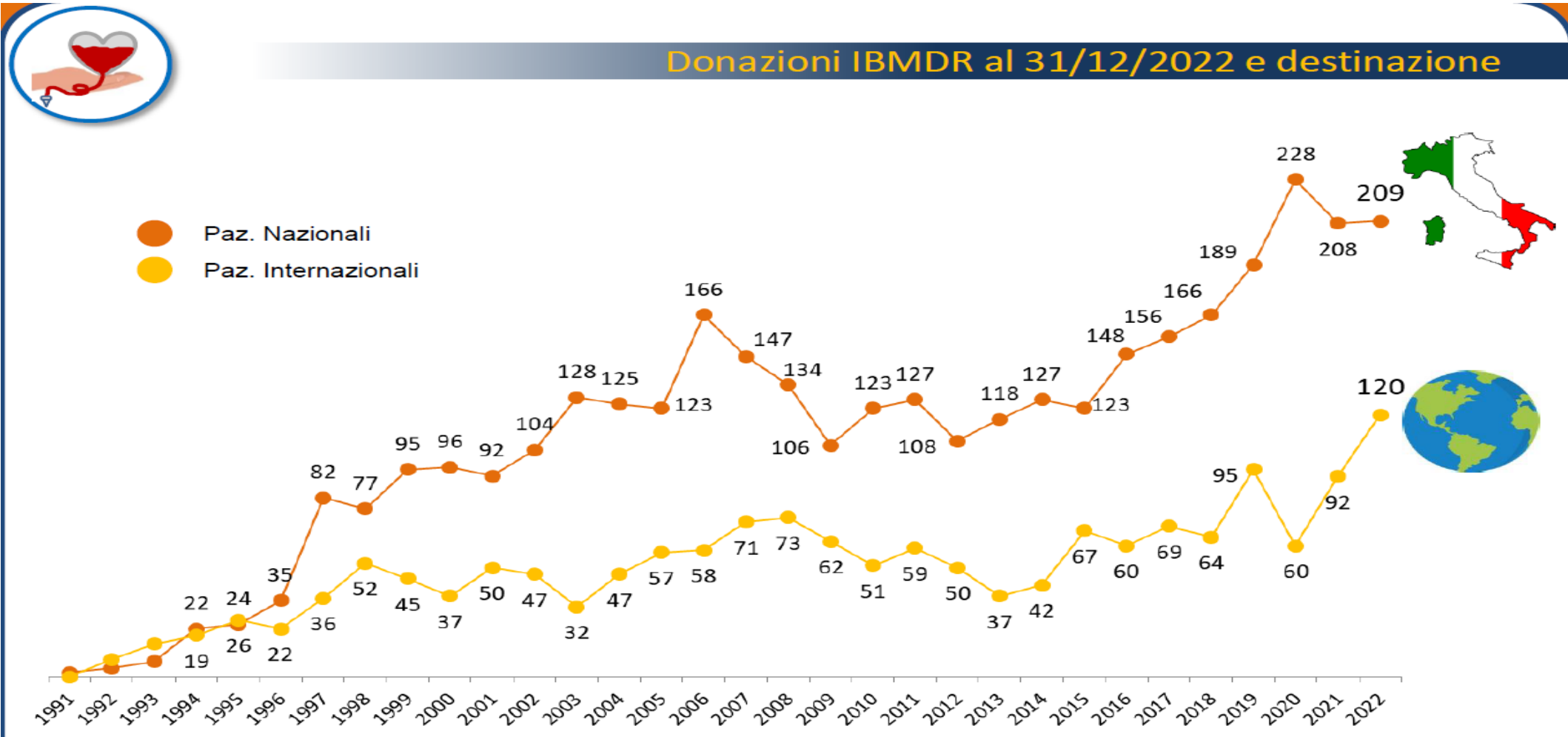
Totale I donazioni 2022: 329



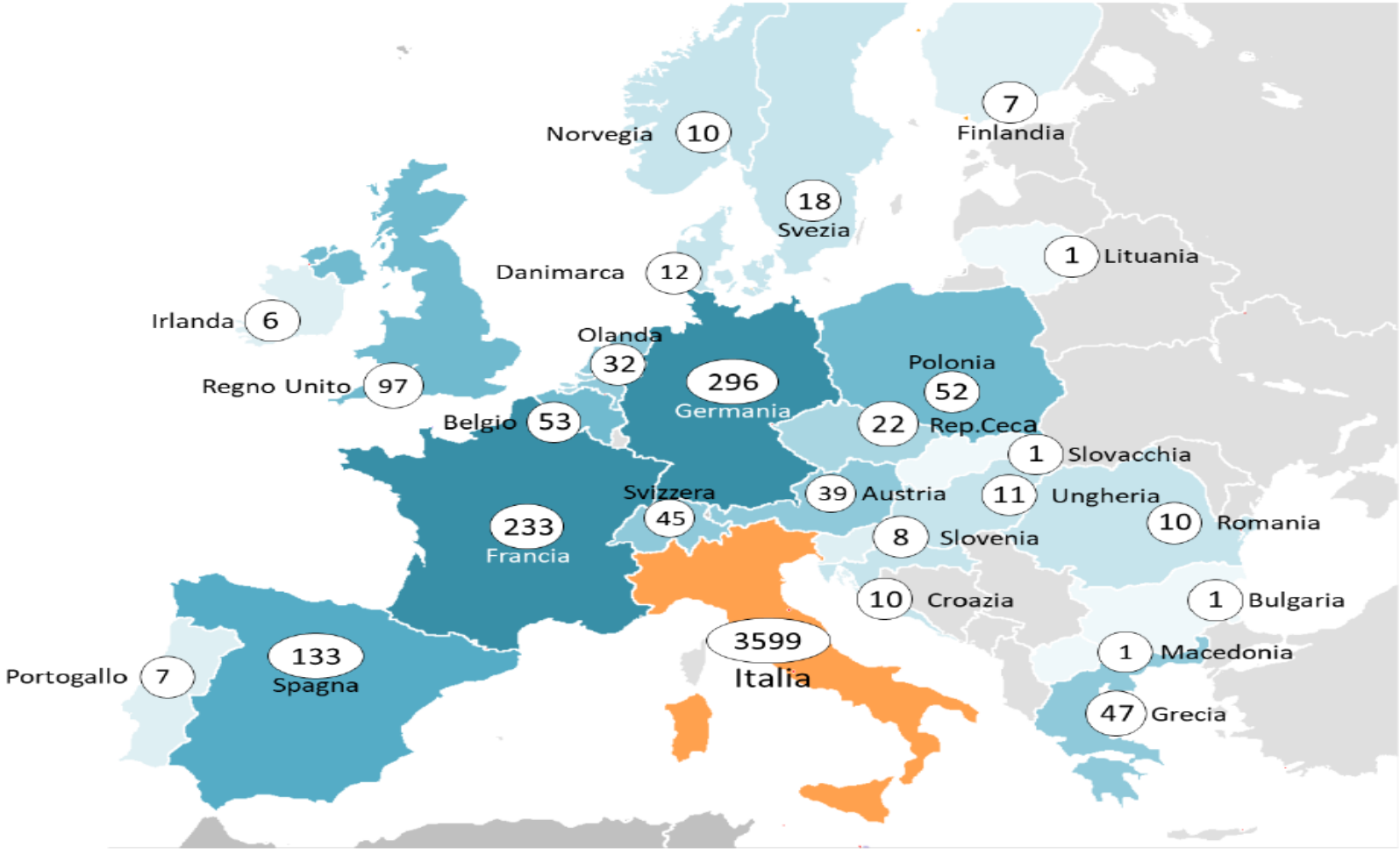




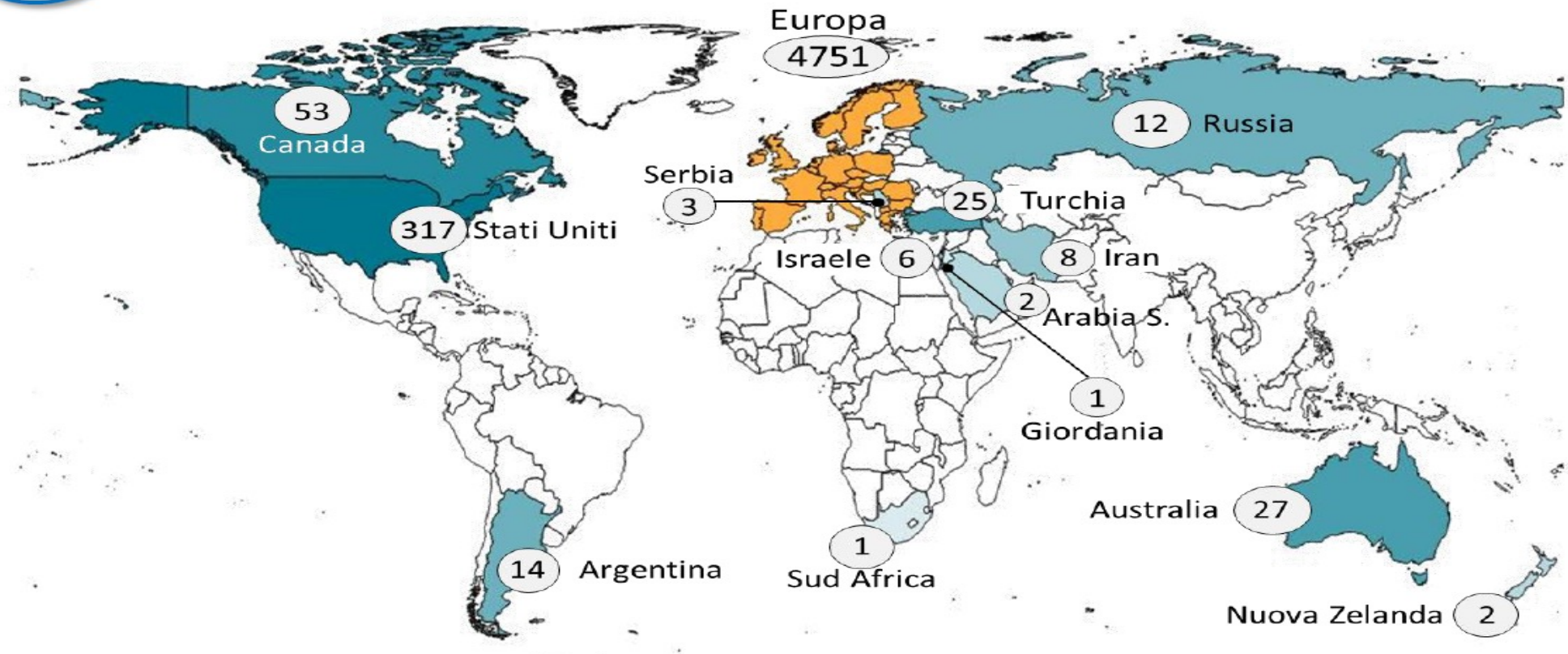
Donazioni IBMDR al 31/12/2022 e destinazione



I donazioni IBMDR - Destinazione in Europa

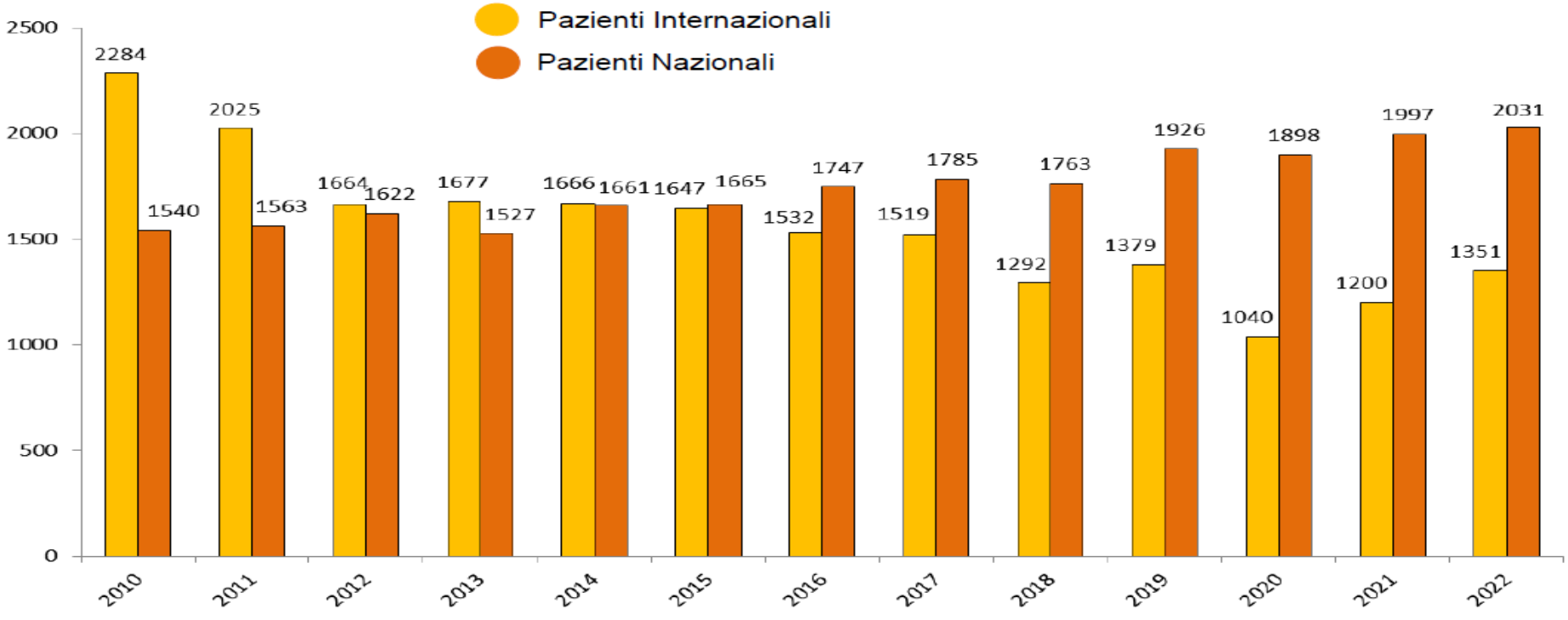


I donazioni IBMDR - Destinazione nel mondo



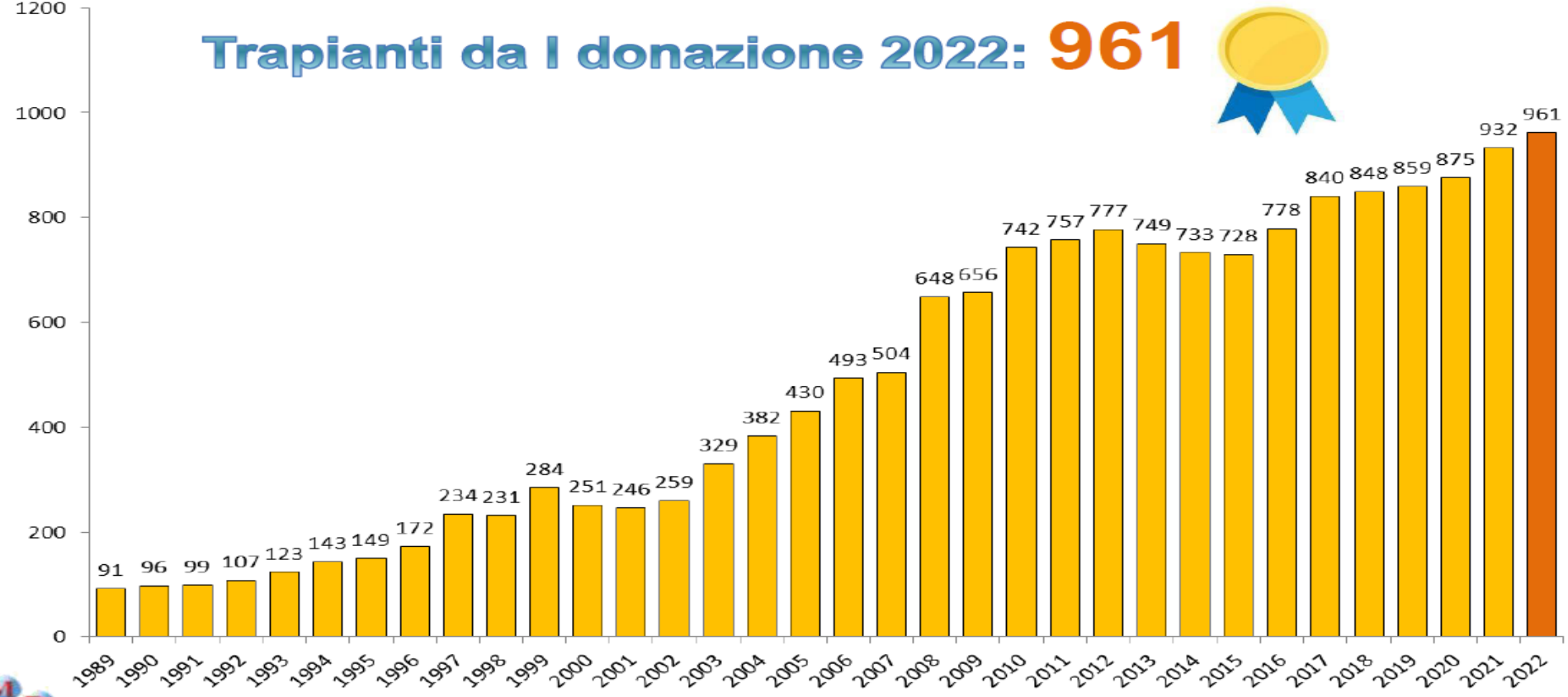
Totale I Donazioni: 5.222

Attivazioni di ricerca

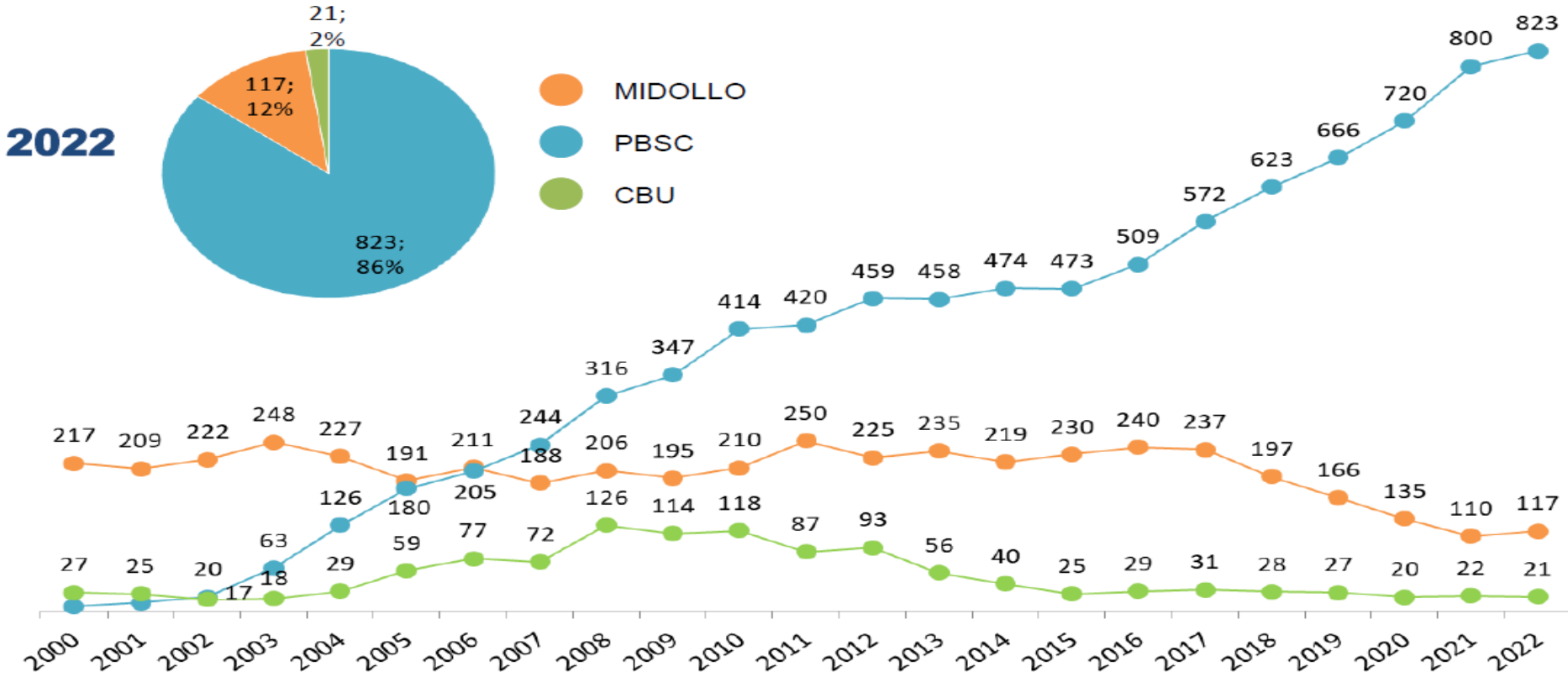


Primi trapianti di CSE da non familiare in Italia

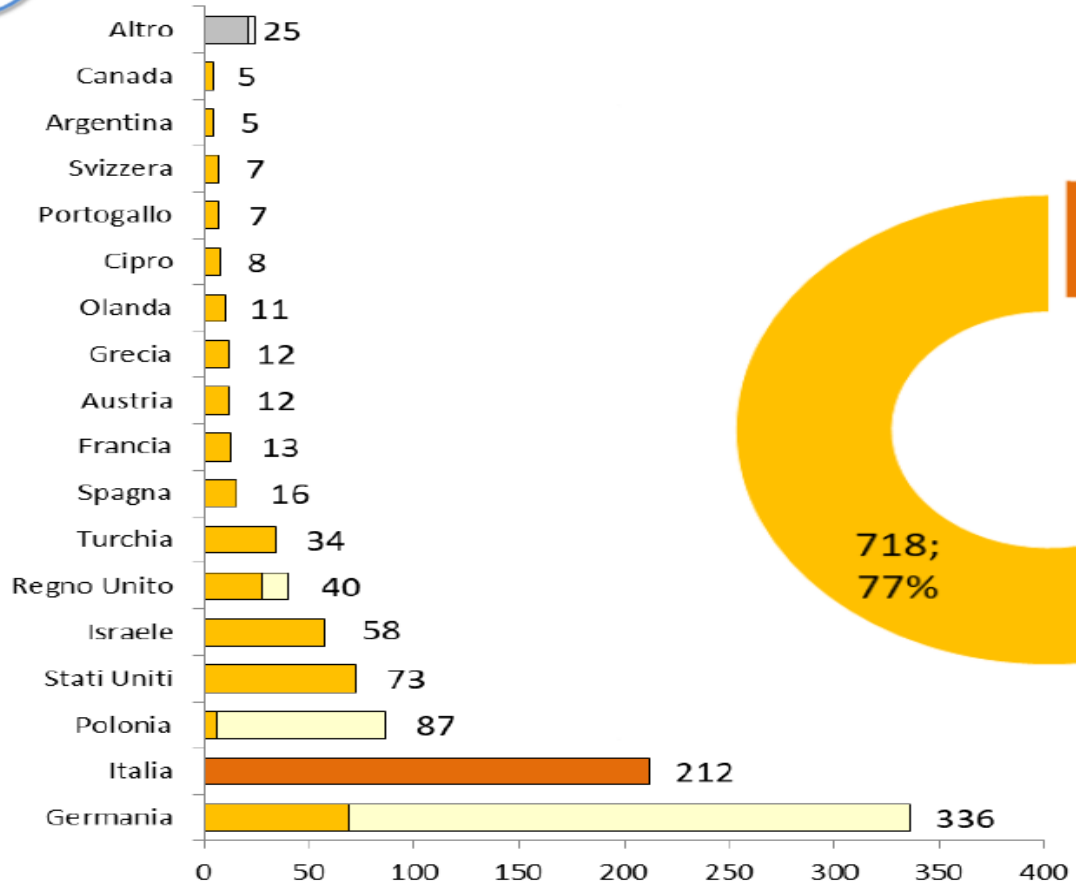
Trapianti da I donazione 2022: 961



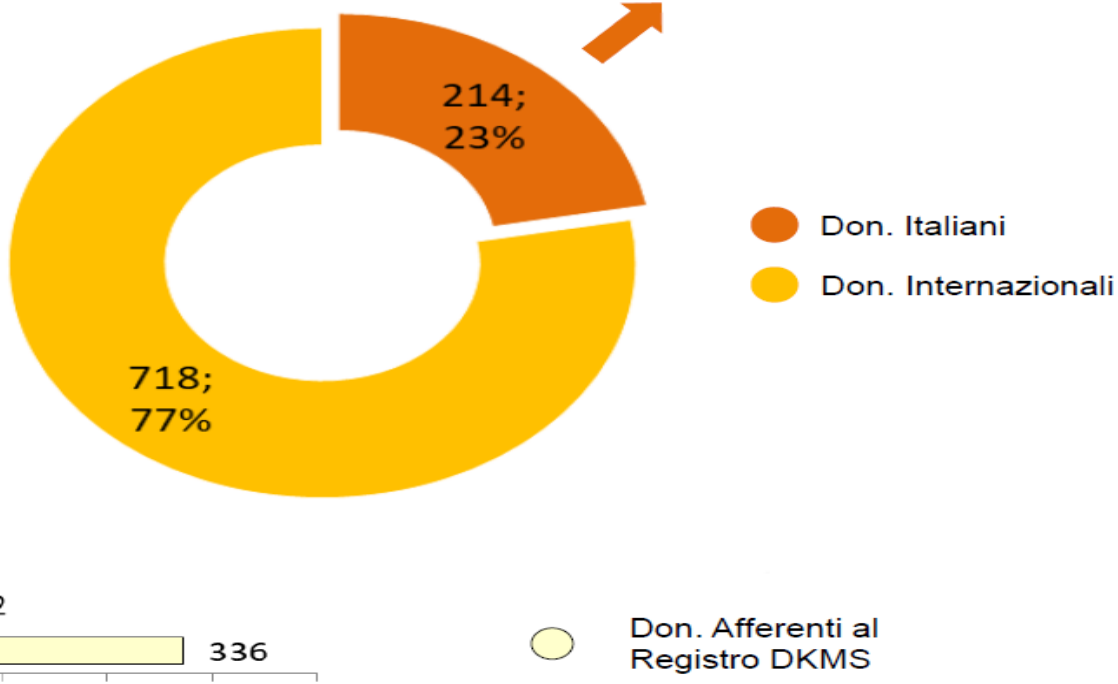
Sorgente CSE nei trapianti da non familiare in Italia



Trapianti 2022: Provenienza CSE

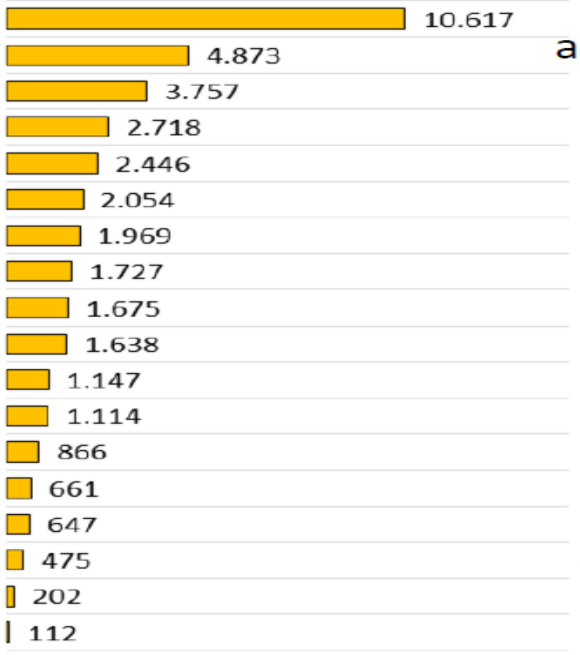


Autosufficienza IBMDR



Inventario unità SCO

CBB	Unità in ITCBN al 31 dicembre 2022
Milano	10.617
Bologna	4.873
Pavia	3.757
Padova	2.718
Firenze	2.446
Torino	2.054
Campania	1.969
Puglia	1.727
Pescara	1.675
Lazio	1.638
Pisa	1.147
Treviso	1.114
Calabria	866
Unicatt	661
Genova	647
Sicilia	475
Sardegna	202
Verona	112
TOTALE	38.698



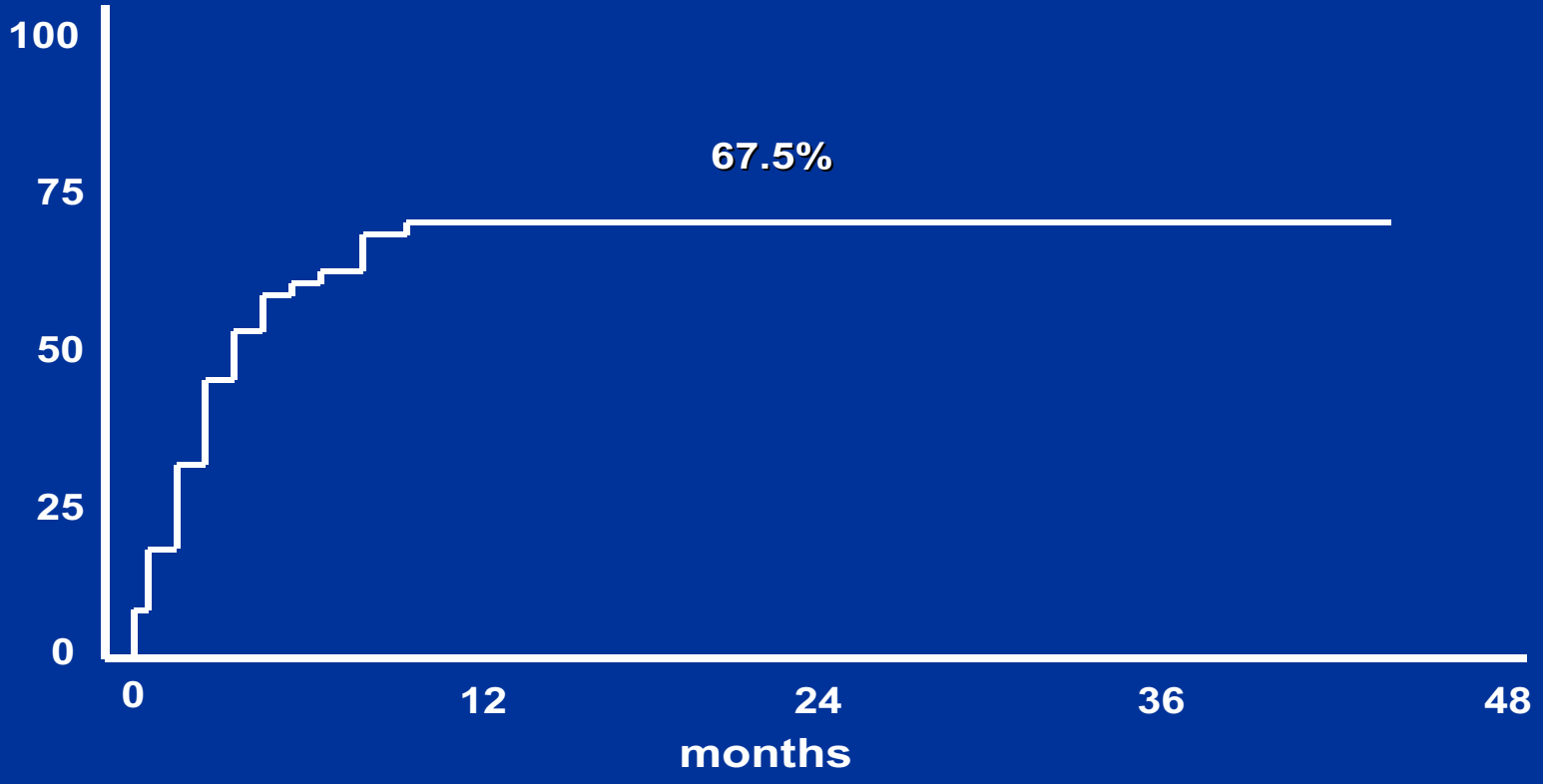
Unità ATTIVE
in ITCBN
al 31 Dicembre 2022:

38.698

Incremento unità:

+365

PROBABILITA' DI IDENTIFICARE UN DONATORE

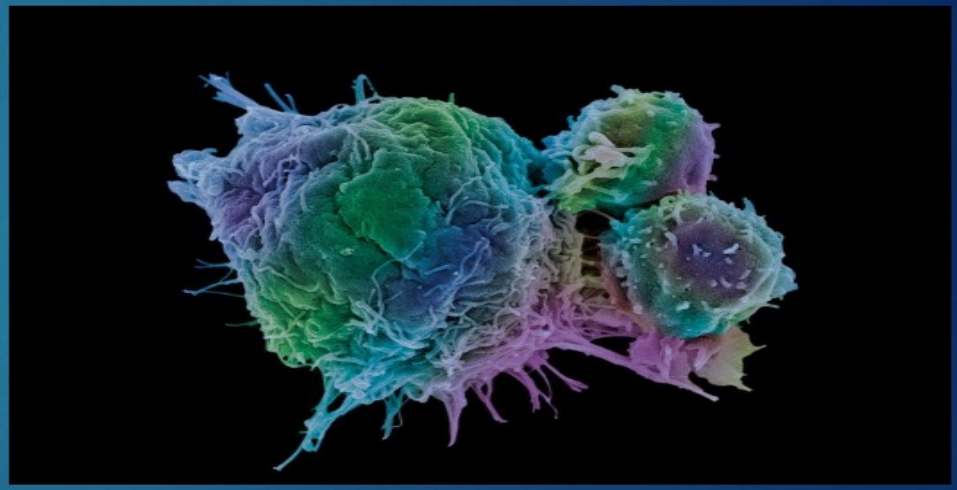


ADMO®
ASSOCIAZIONE DONATORI
MIDOLLO OSSEO

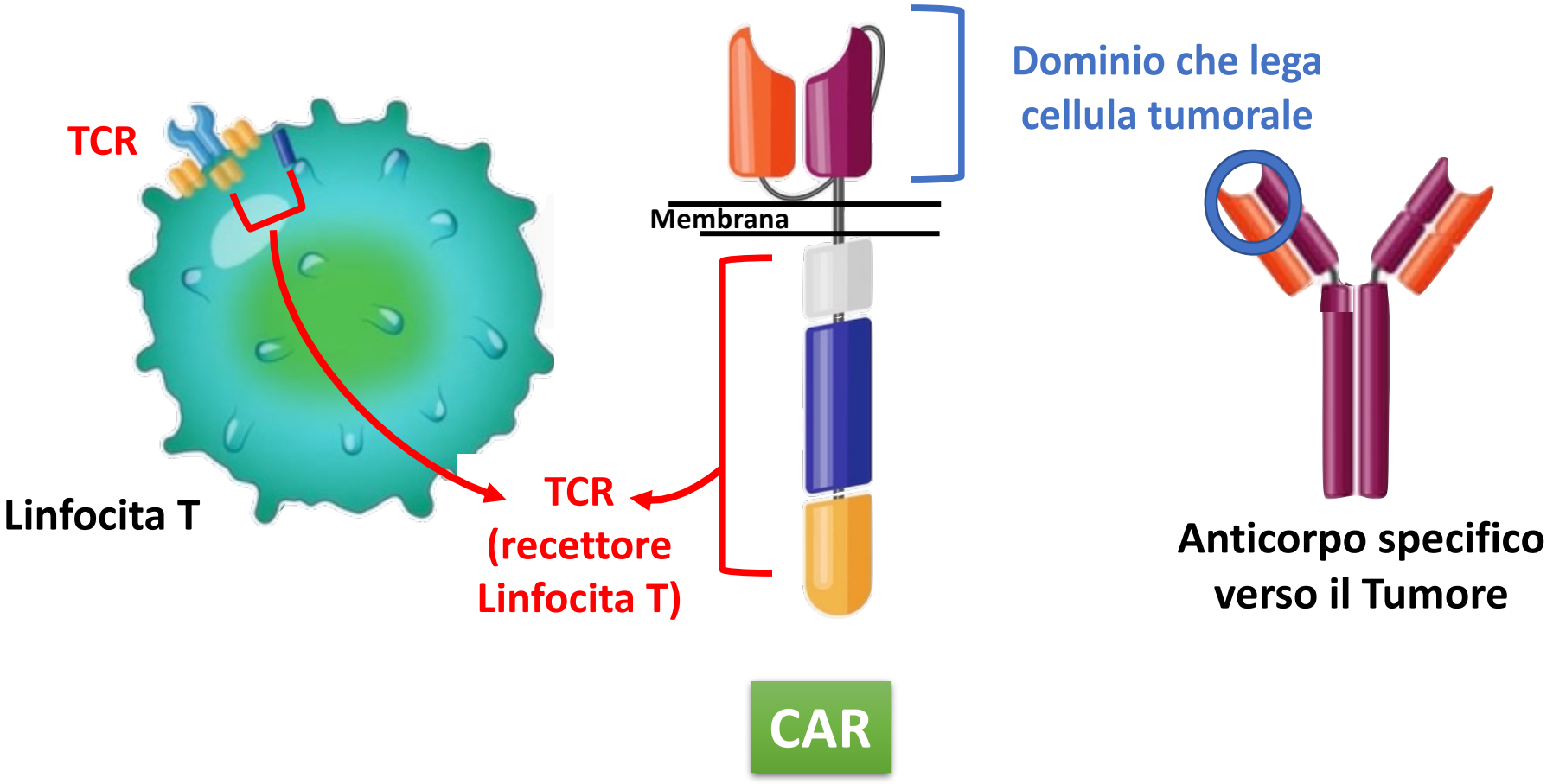
PENSACI...ABBIAMO BISOGNO ANCHE DI TE!!!

Chimeric Antigen Receptor T-Cells (CAR-T):

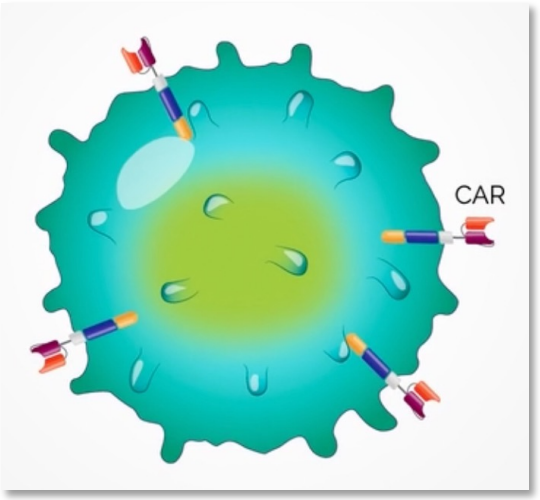
what are
and
how they work



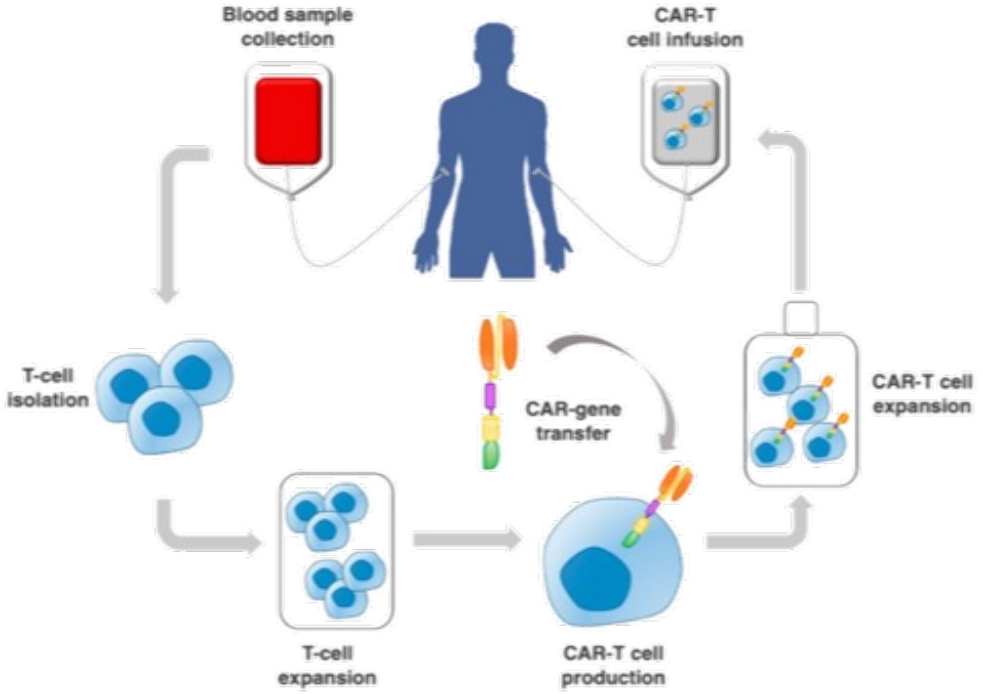
Chimeric Antigen Receptor



CAR T-Cell Therapy Fights Cancer with a Live Weapon

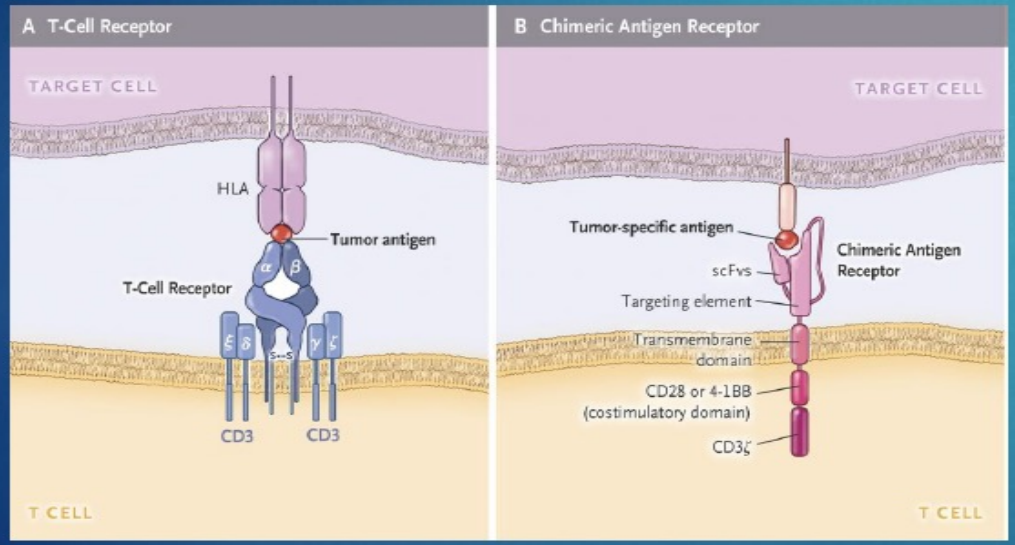


CAR-T cells

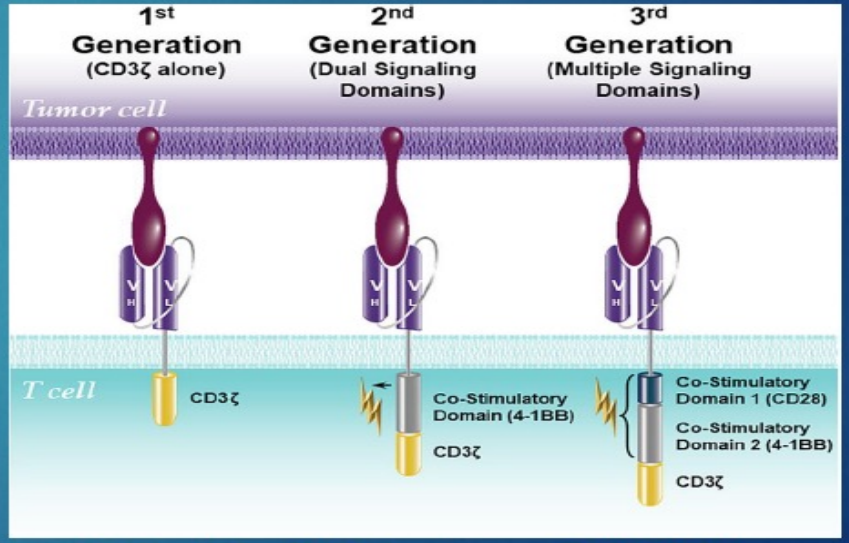


What are CAR-T Cells

- genetically redirect and reprogram T cells to overcome tolerance in cancer;
- patient's own T cells genetically engineered with gene encoding chimeric antigen receptor (CAR) that binds a tumor antigen;
- CARs consist of:
  - extracellular immunoglobulin-derived variable heavy and light chains to direct specificity;
  - intracellular signaling molecule comprised of TCR CD3 zeta chain;
  - co-stimulation signaling, such as CD28 and 4-1BB, that increases expansion and potency of engineered T cells.



Structure of CARs and T-Cell Receptors.
June CH, Sadelain M.
N Engl J Med 2018;379:64-73



An introduction to chimeric antigen receptor (CAR) T-cell immunotherapy for human cancer
Feins S. et al.
Am J Hematol. 2019;94:S3–S9

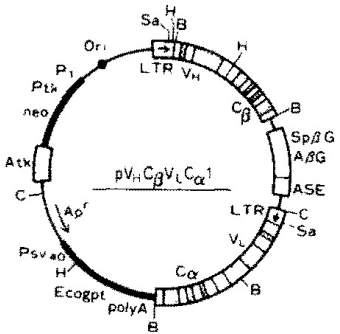
Different CAR generation

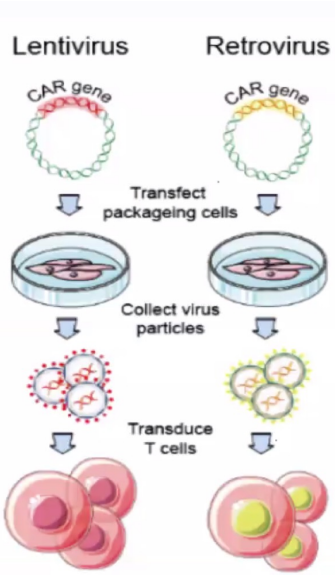
Vol. 149, No. 3, 1987
December 31, 1987

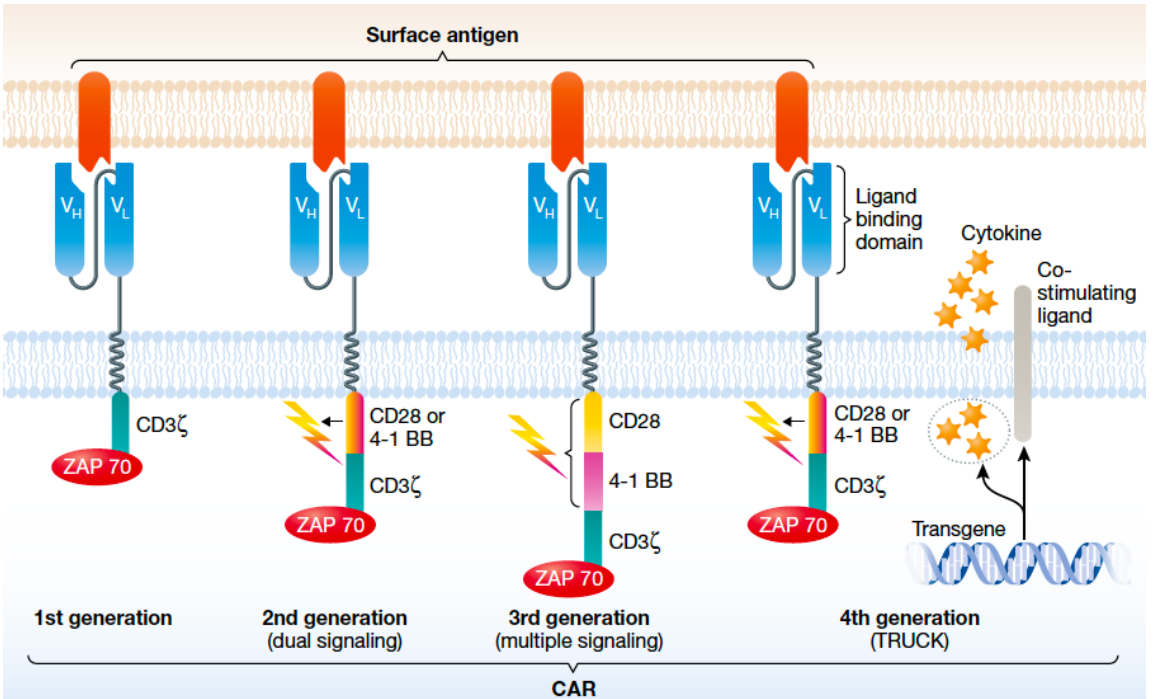
BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 960-968

EXPRESSION OF CHIMERIC RECEPTOR COMPOSED OF IMMUNOGLOBULIN-DERIVED V RESIONS AND T-CELL RECEPTOR-DERIVED C REGIONS

Yoshihisa Kuwana[1], Yoshihiro Asakura[1], Naoko Utsunomiya[2], Mamoru Nakanishi[2], Yohji Arata[2], Seiga Itoh[3], Fumihiko Nagase[4] and Yoshikazu Kurosawa[1]*







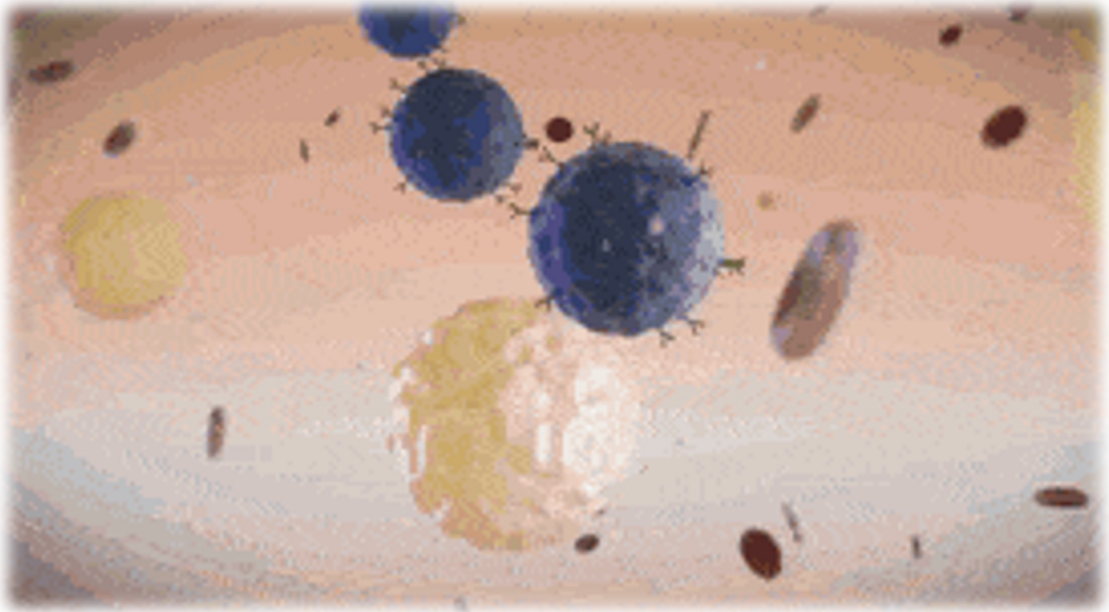
Cytotoxicity

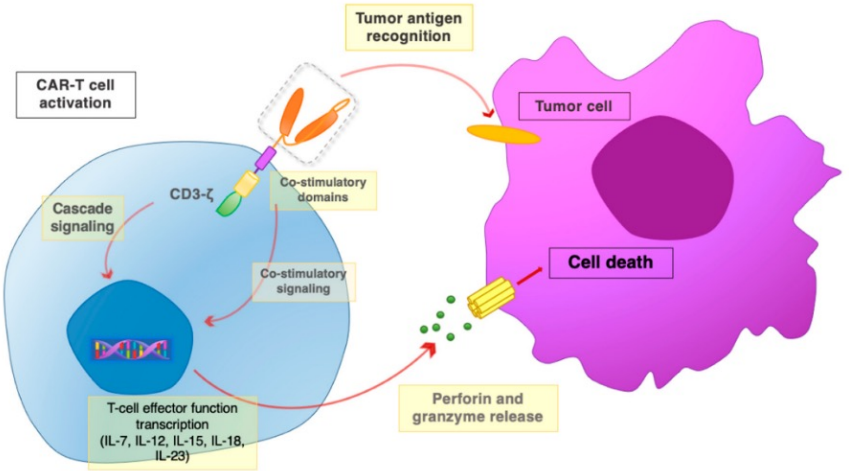
Proliferation/cytokine production

Survival

Kuwana et al. Biochem Biophys Res Commun 1987; Gross et al. Transplant Proc 1989; Hartmann et al., EMBO Mol Med. 2017

CAR T-Cell Therapy Fights Cancer with a Live Weapon





Killing mechanisms of CAR-T cells:

- **Perforin and granzyme axis**: *targeting antigen positive fraction*
- **Cytokine secretion**: *stromal cell sensization*
- **Fas and FasL axis**: *targeting antign-negative fraction*

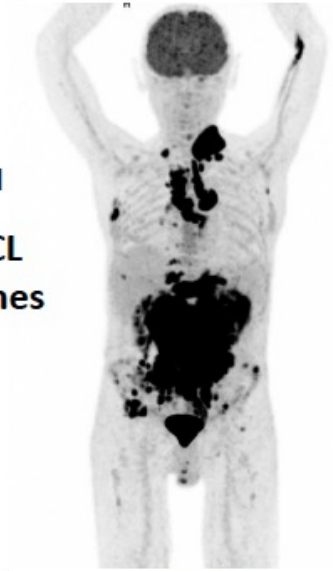
Benmebarek et al., IJMS 2019

Emerging immunotherapies will enhance the immune response against tumor cells

To use the own immunity of the patient to cure cancer

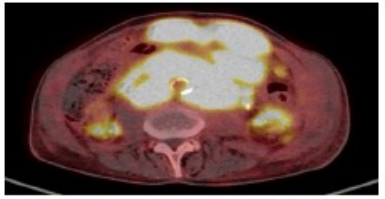
Case report
THE, male, 63 years old

- R/R DLBCL
- 3 prior lines

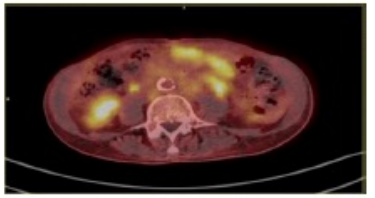


CAR T-cells

CURE

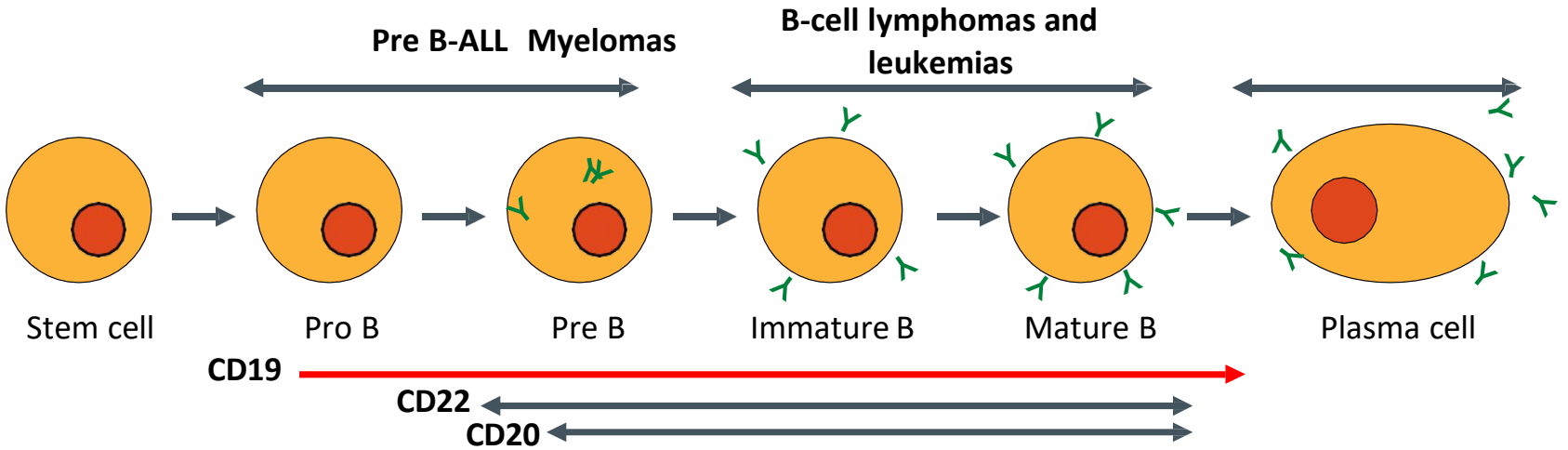


Metabolic tumoral volume 1200ml



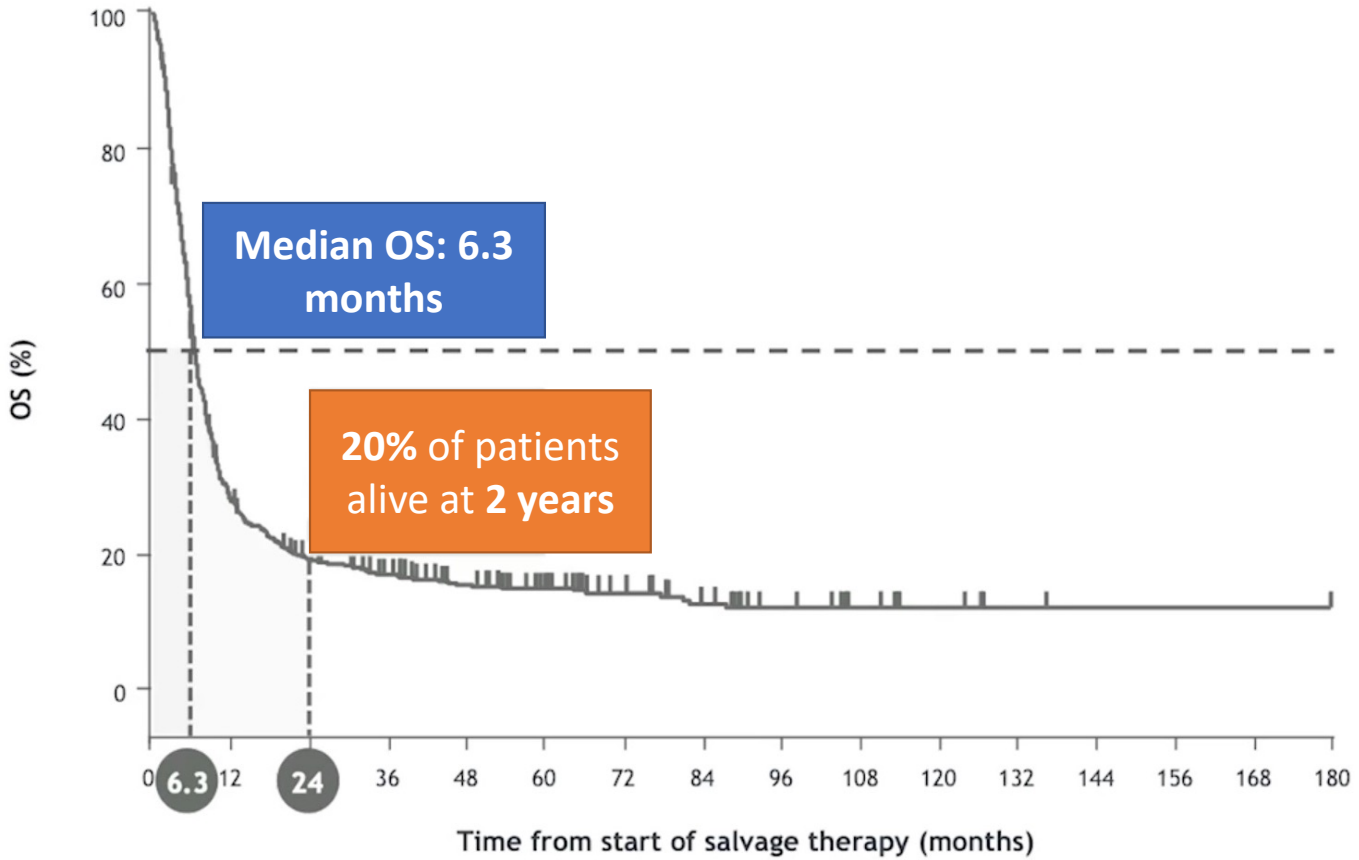
CD19 expression

- CD19 expression is restricted to B-cells and possibly follicular dendritic cells
- CD19 is not expressed on pluripotent bone marrow stem cells
- CD19 is expressed on the surface of most B-cell malignancies
- Antibodies against CD19 inhibit growth of tumor cells



Mostolizadeh. Numer Algebra Control Optim. 2018;8:63. Wang. Exp Hematol Oncol. 2012;1:36.

Scholar 1 study in r/r DLBCL patients



SCHOLAR-1
(N=636)

Included patients with:

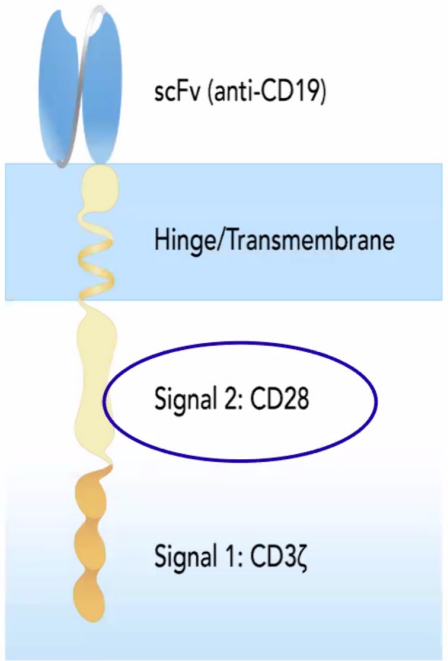
- **Primary refractory disease**
- **Refractory to ≥2 lines of therapy**
- **Relapse ≤12 months post ASCT**

Crump et al., Blood 2017

Pivotal phase II DLBCL Trials (≥ 2 lines of therapy)

ZUMA 1 trial

Axicabtagene Ciloleucel (Axi-cel)

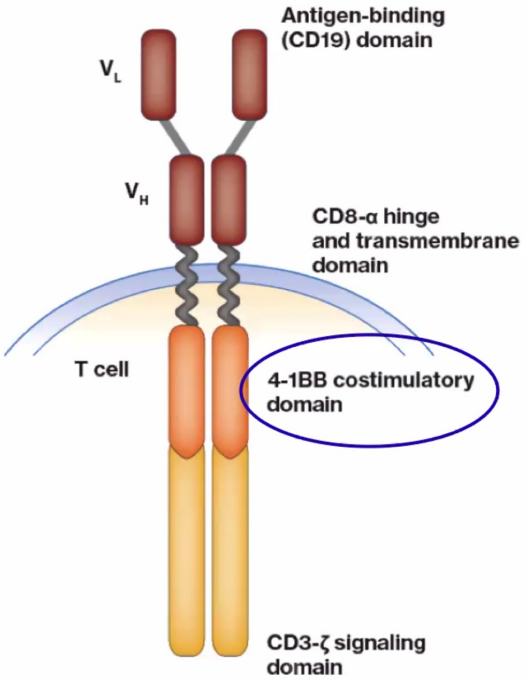


Vector: Retrovirus

Starting material: PBMCs

JULIET trial

Tisagenlecleucel (Tisagen)

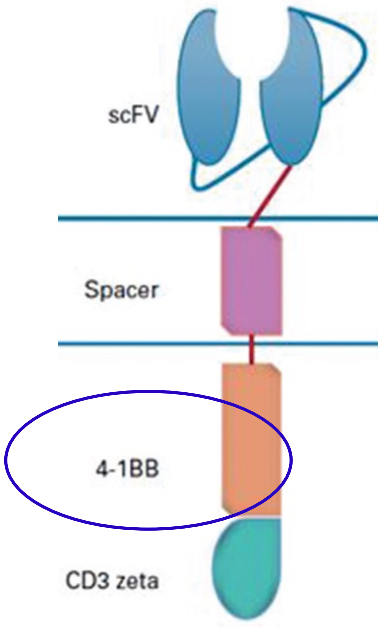


Vector: Lentivirus

Starting material: $CD3^+$

TRASCEND-NHL-001 trial

Lisocabtagene maraleucel

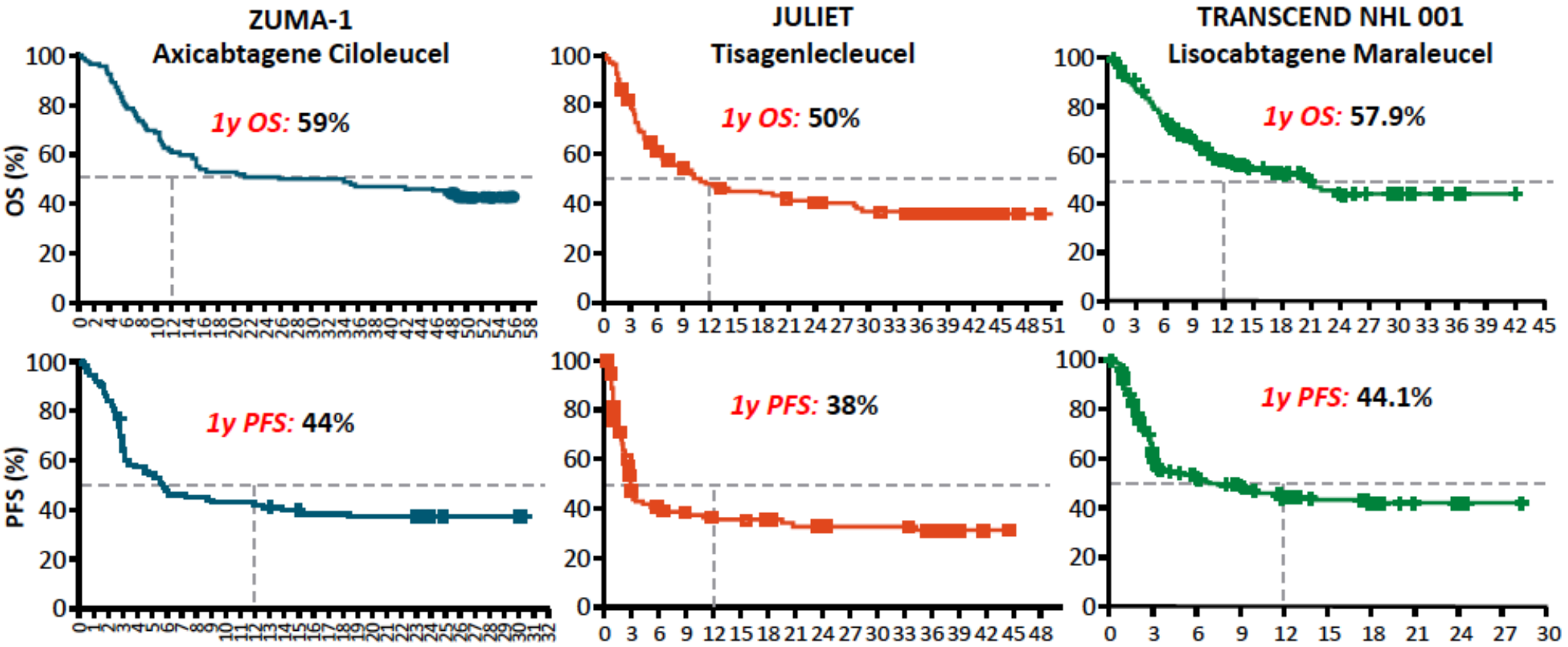


Vector: Lentivirus

Starting material: CD4/CD8

Locke et al., Lancet Oncol, 2019; Schuster et al., NEJM 2019; Abramson et al., Lancet 2020

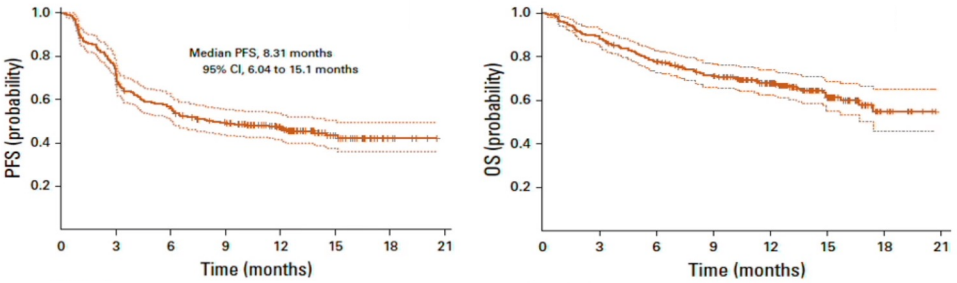
Pivotal Anti CD19 CAR T Cell Therapy Trials in DLBCL



Locke. Lancet Oncol. 2019;20:31. Jacobson. ASH 2020. Abstr 1187. Jaeger. ASH 2020. Abstr 1194. Abramson. Lancet. 2020;396:8

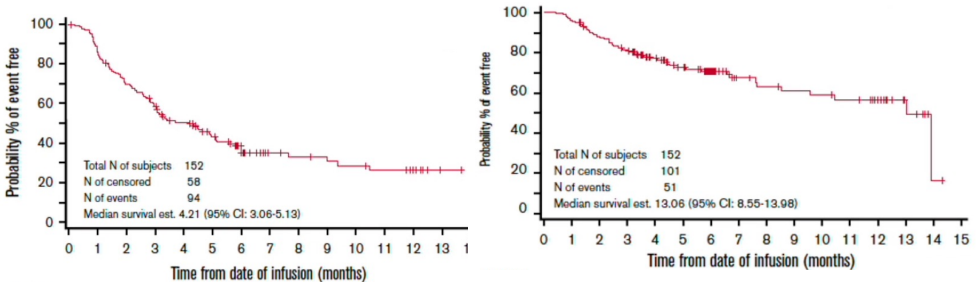
Real Life experiences outcomes

Axi-cel

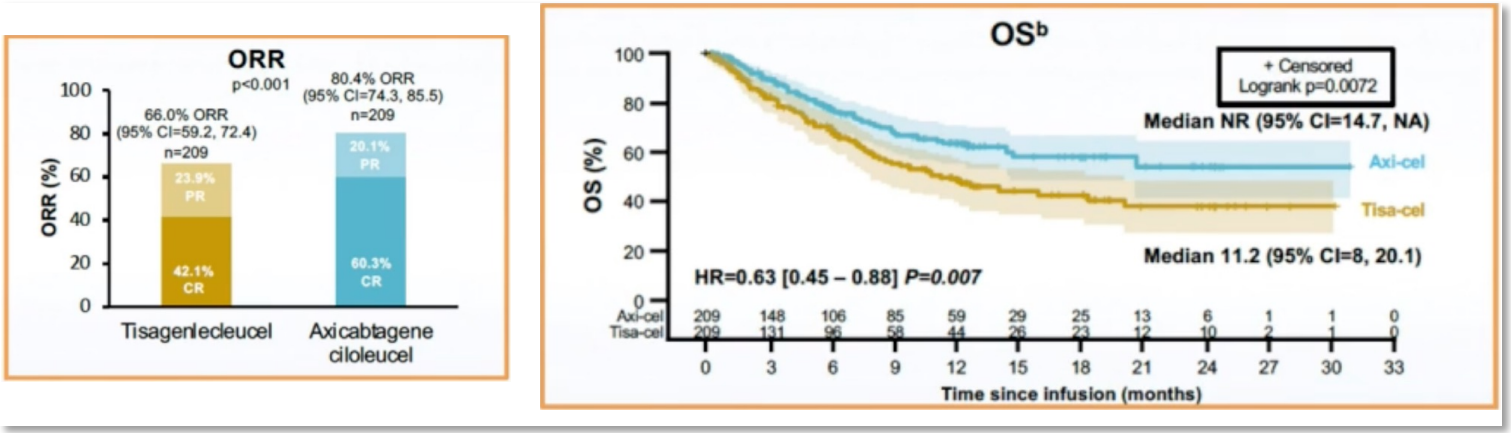


Nastoipil et al., JCO 2020

Tisa-cel



Pasquini et al., Blood Adv 2020



Bachy et al. Nat Med 2022

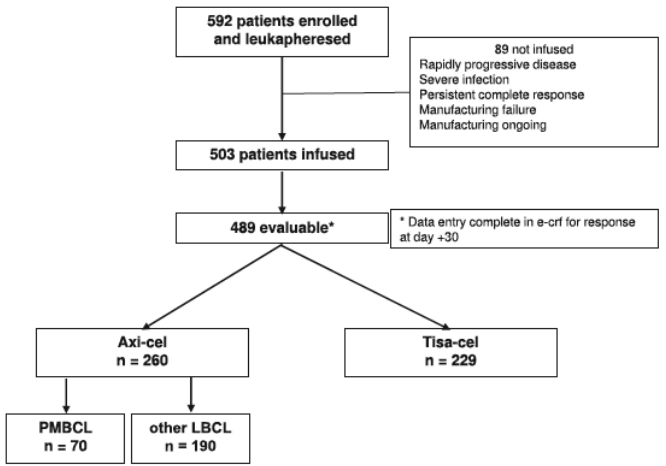
Italian Real life experience

ARTICLE OPEN

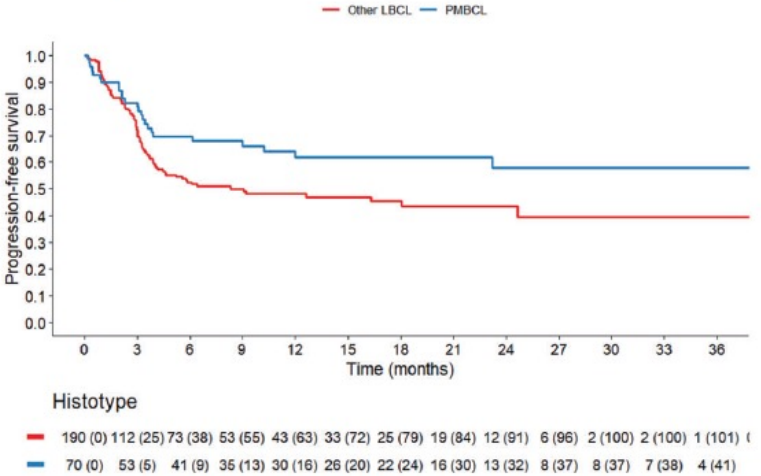
LYMPHOMA

Axicabtagene ciloleucel treatment is more effective in primary mediastinal large B-cell lymphomas than in diffuse large B-cell lymphomas: the Italian CART-SIE study

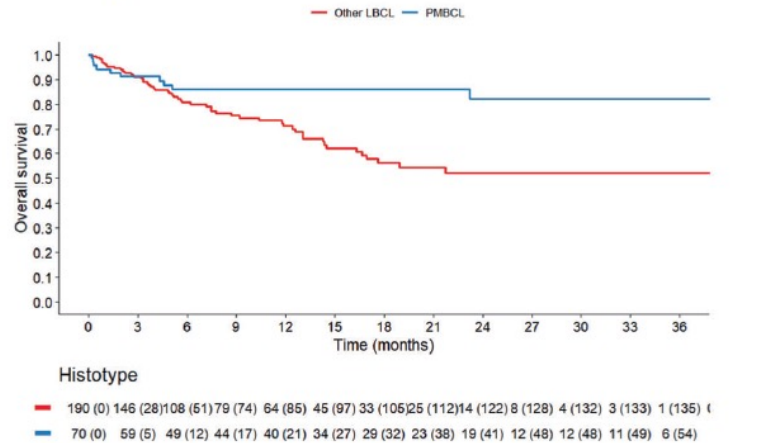
Annalisa Chiappella[1,23], Beatrice Casadei[2], Patrizia Chiusolo[3], Alice Di Rocco[4], Silva Ljevar[5], Martina Magni[1], Piera Angelillo[6], Anna Maria Barbui[7], Ilaria Cutini[8], Anna Dodero[1], Francesca Bonifazi[2], Maria Chiara Tisi[9], Stefania Bramanti[10], Maurizio Musso[11], Mirko Farina[12], Massimo Martino[13], Mattia Novo[14], Giovanni Grillo[15], Francesca Patriarca[16], Giulia Zacchi[17], Mauro Krampera[18], Martina Pennisi[1], Eugenio Galli[3], Maurizio Martelli[4], Andrés J. M. Ferreri[6], Silvia Ferrari[7], Riccardo Saccardi[8,21], Anisa Bermema[1], Anna Guidetti[1,19], Rosalba Miceli[5], Pier Luigi Zinzani[2,20] and Paolo Corradini[1,19]



PFS

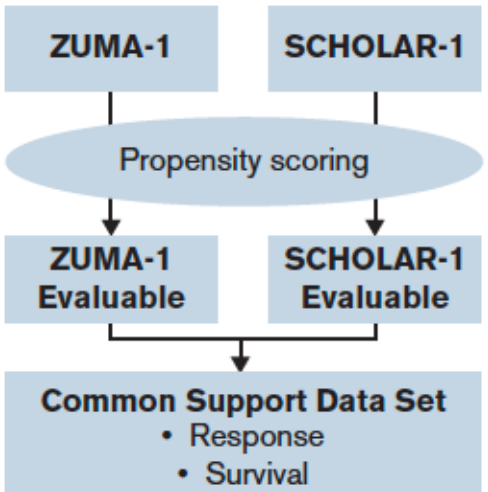


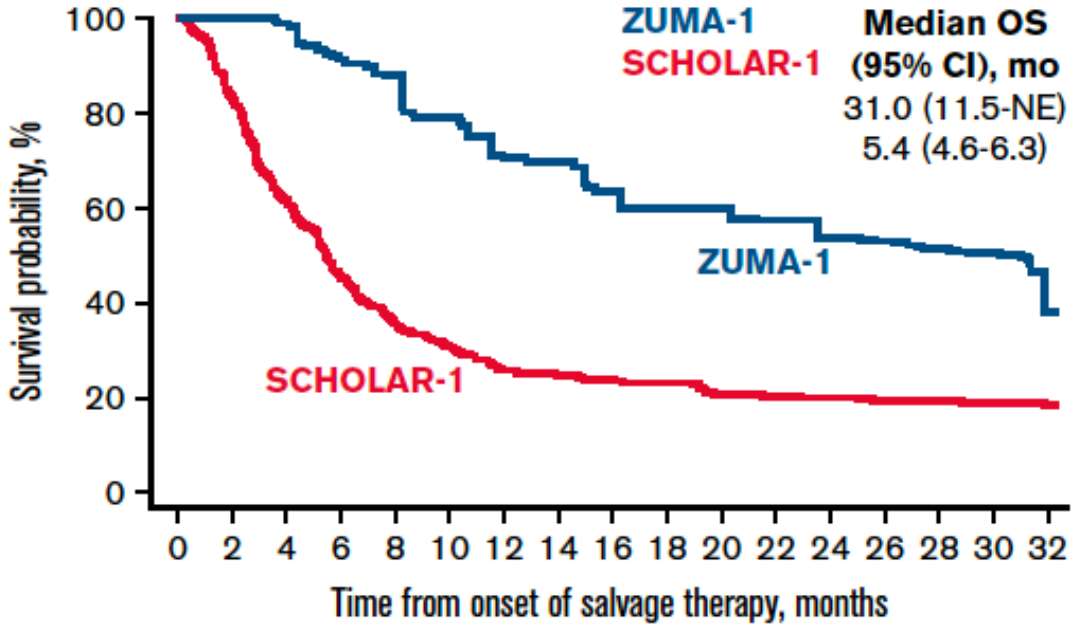
OS



Chiappella et al., Leukemia 2024

Comparison of 2ys outcome with ZUMA-1 vs. salvage chemotherapy in refractory DLBCL

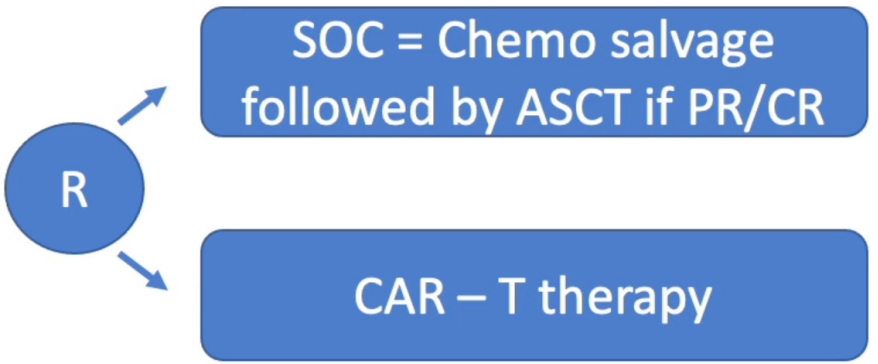




	ZUMA-1	SCHOLAR-1	Ratio
12-mo OS rate (95% CI), %	71 (46-91)	26 (22-32)	2.7 (1.7-3.8)
24-Mo OS rate (95% CI), %	54 (30-80)	20 (16-26)	2.7 (1.4-4.3)
Median OS (95% CI), mo	31 (11.5-NE)	5.4 (4.6-6.3)	25.6 (6.0-NE)

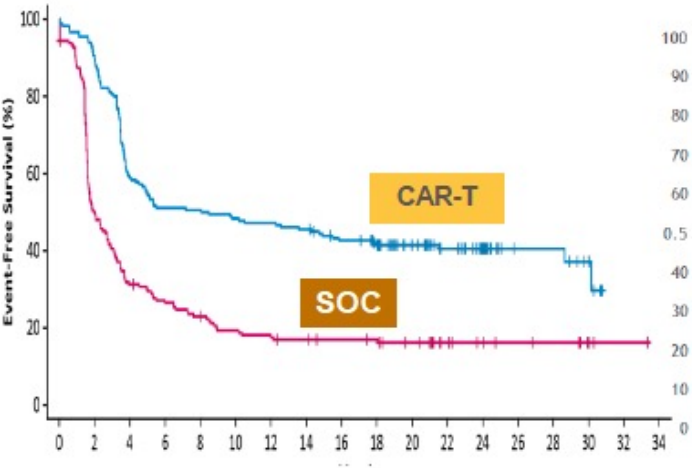
Neelapu et al., Blood Adv 2021

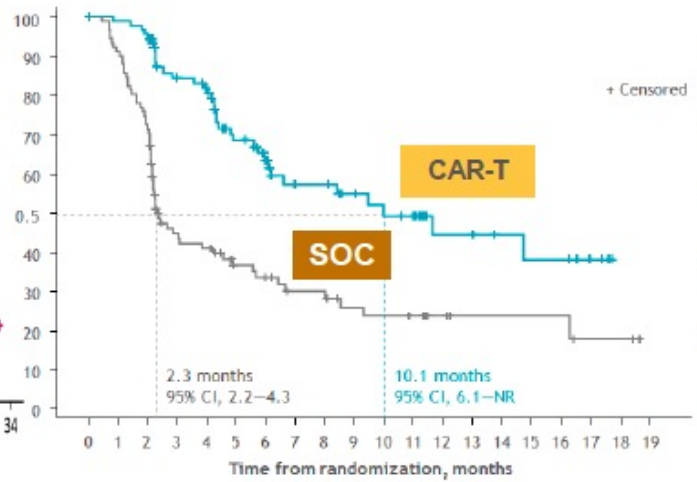
Can CAR-T cell beat autoSCT in 2nd line?

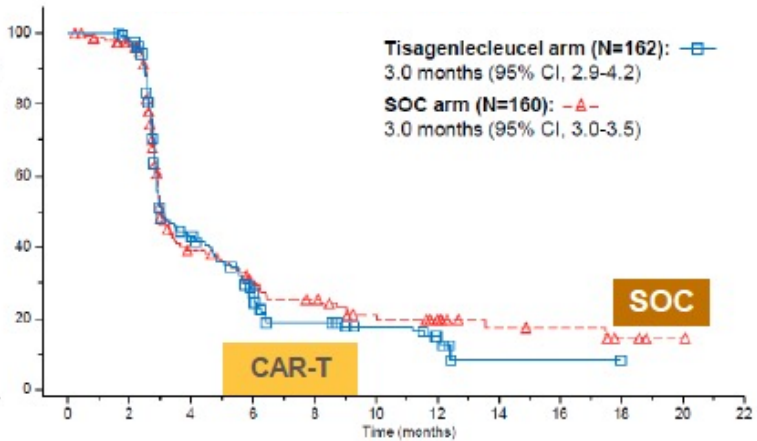


Axi-cel	**ZUMA-7**
Liso-cel	**TRANSFORM**
Tisa-cel	**BELINDA**

Randomized Ph3 Studies (*CAR-T vs. SOC ASCT*): EFS







ZUMA-7	Transform	BELINDA
Median EFS = 8.3 vs. 2 mons	Median EFS = 10.1 vs. 2.3 mons	Median EFS = 3 vs. 3 mons
1. Progression or death 2. New treatment 3. No CR/PR by 150 days	1. Progression or death 2. New treatment 3. No CR/PR by 9wks	1. Progression or death 2. SD/PD @/after 12wks
ORR: 83% vs. 50% **CR: 65% vs. 32%**	**ORR: 86% vs. 48%** **CR: 66% vs. 39%**	**ORR: 46.3% vs. 42.5%** **CR: 28.4% vs. 27.5%**

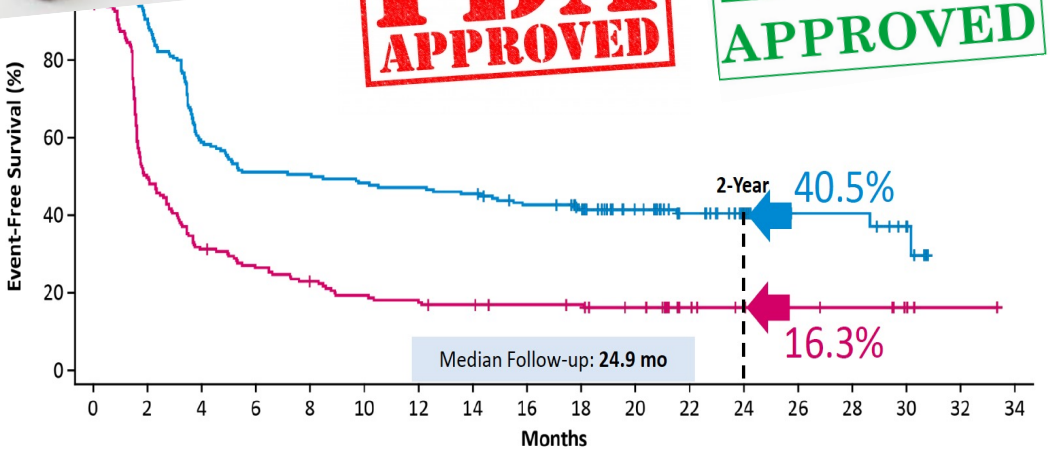
Locke et al., NEJM 2022, Kamdar et al., Lancet 2022; Bishop et al., NEJM 2022

Primary EFS endpoint: Axi-cel and Lisa-cel are superior to SOC

Median FU: 24.9 months

FDA APPROVED

EMA APPROVED

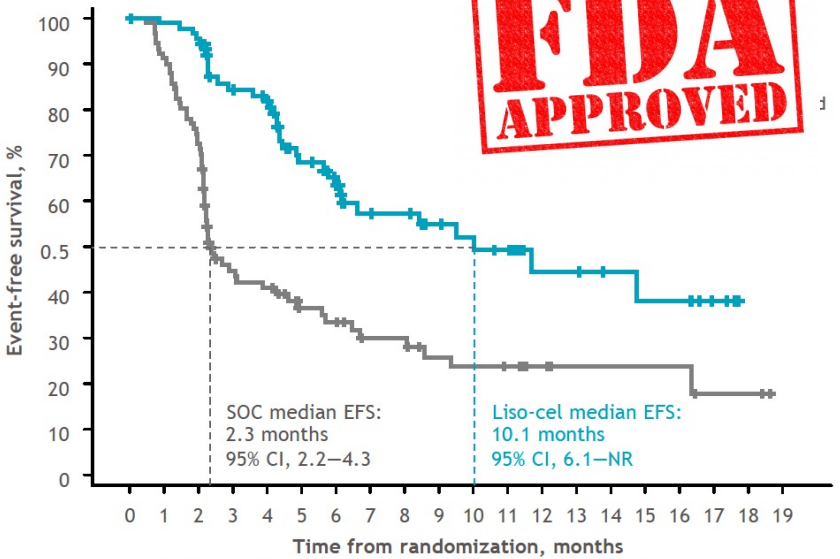


HR 0.398 (95% CI, 0.308-0.514); *P*<0.0001

	Median EFS (95% CI), mo	24-mo EFS Rate (95% CI), %
Axi-cel (N=180)	8.3 (4.5-15.8)	40.5% (33.2-47.7)
SOC (N=179)	2.0 (1.6-2.8)	16.3% (11.1-22.2)

Median FU: 6.2 months

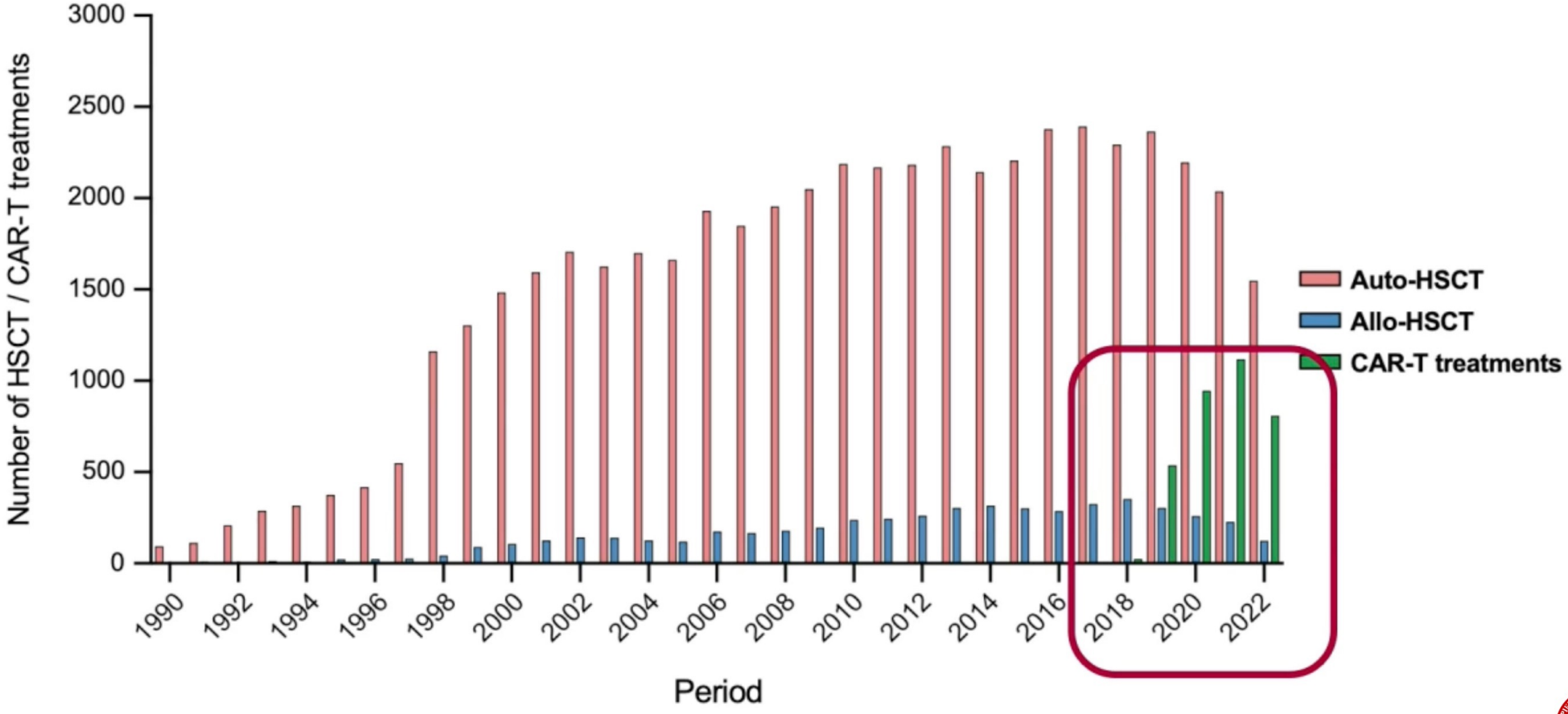
FDA APPROVED



	Liso-cel arm (n = 92)	SOC arm (n = 92)
Patients with events, n	35	63
Stratified HR (95% CI)	0.349 (0.229—0.530) *P* < 0.0001	
6-month EFS rate, % (SE)	63.3 (5.77)	33.4 (5.30)
Two-sided 95% CI	52.0—74.7	23.0—43.8
12-month EFS rate, % (SE)	44.5 (7.72)	23.7 (5.28)
Two-sided 95% CI	29.4—59.6	13.4—34.1

Locke et al., NEJM 2022; Kamdar et al., ASH 2021

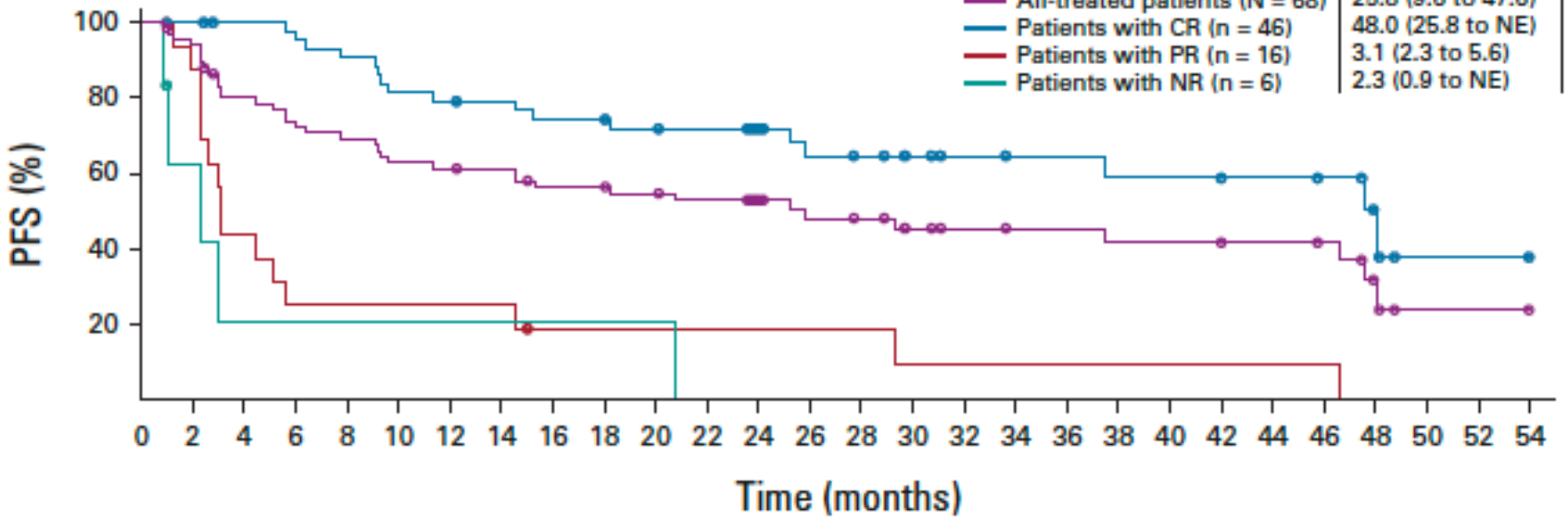
HCT and CART cell activity over time for DLBCL _ EBMT registry



Current Status of CD19-CAR-T: Mantle Cell Lymphoma

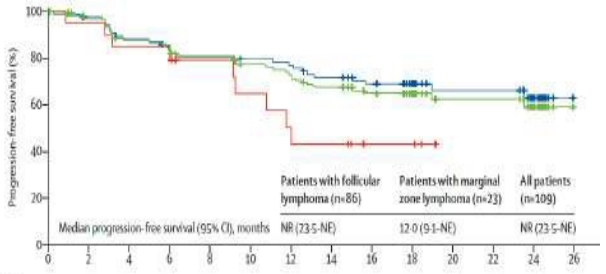
Brexu-cel; ZUMA 2

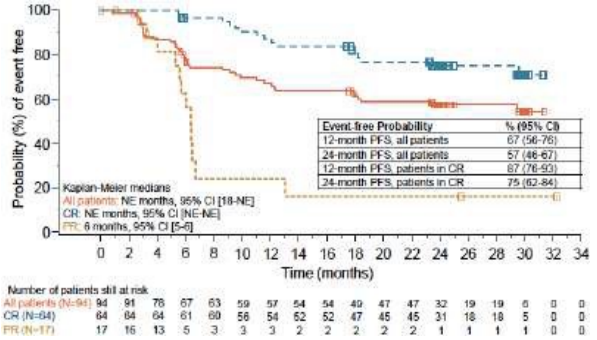
	Median PFS, Months (95% CI)	24-Month PFS Rate, % (95% CI)
All-treated patients (N = 68)	25.8 (9.6 to 47.6)	52.9 (39.9 to 64.3)
Patients with CR (n = 46)	48.0 (25.8 to NE)	71.8 (55.7 to 82.9)
Patients with PR (n = 16)	3.1 (2.3 to 5.6)	18.8 (4.6 to 40.2)
Patients with NR (n = 6)	2.3 (0.9 to NE)	ND

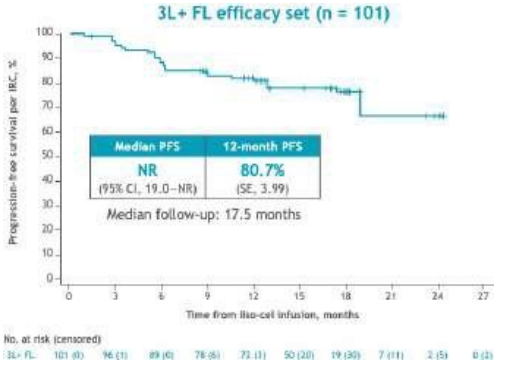


Wang, et al. JCO 2023

Current Status of CD19-CAR-T: Follicular Lymphoma







	Axicabtagene Ciloleucel ZUMA-5	Tisagenlecleucel Elara	Lisocabtagene Maraleucel TRANSCEND-FL
n	124	97	107
Median # prior lines	3	4	3
Chemorefractory	68%	78%	67%
POD24	55%	60%	54%
CR rate	79%	68%	94%
Median PFS, m	NR	NR	NR
PFS	65% at 18m	57% at 24m	81% at 12m
CRS (Any/severe) %	82/7	49/0	58/1
NT (Any/severe) %	59/19	4/1	15/2

Jacobson, et al. Lancet Onc 2022; Dreyling, at al. Proc ASH 2022 Fowler, et al. Nat Med 2022.; Morschhauser, et al. Proc ICML 2023

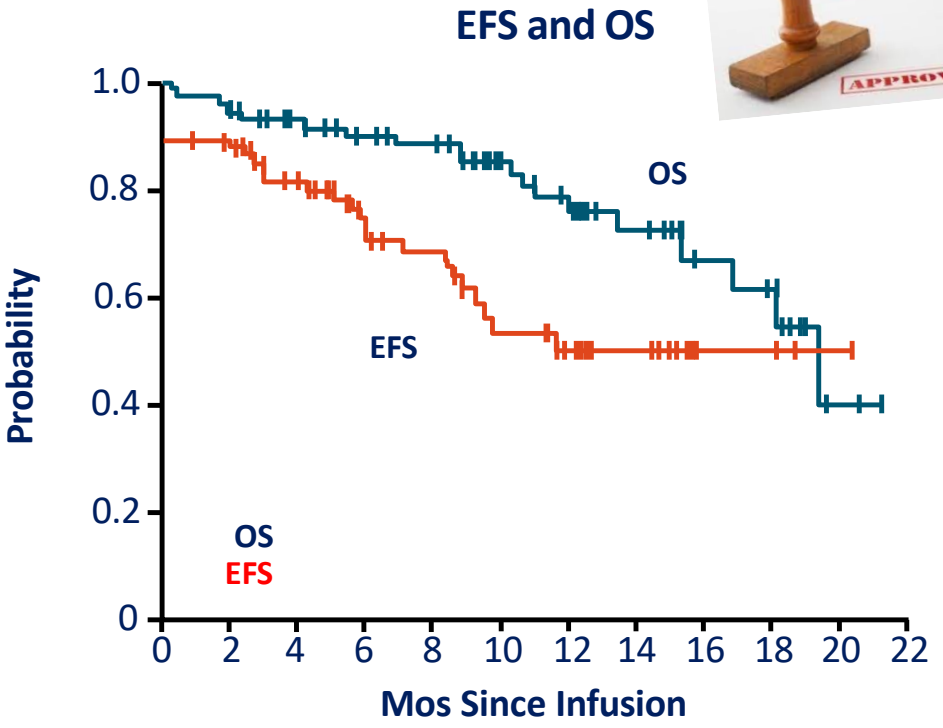
AIFA indications_Lymphoma

- DLBCL (18-75 anni)
  - Axi-cel:
    - II linea in refrattari e recidiva < 12 mesi (Autunno 2023)
    - Dalla III linea in poi
  - Tisa-cel:
    - Dalla III linea in poi
  - Liso-cel (Febbraio 2024):
    - Dalla III linea in poi
- PMBCL (18-75 anni)
  - Axi-cel: dalla III linea in poi
  - Liso-cel (Febbraio 2024): dalla III linea in poi
- MCL (> 18 anni)
  - Brexu-cel: dalla III linea in poi (almeno un BTKi)
- FL (> 18 anni)
  - Axi-cel: dalla 4^ linea in poi
  - Tisa-cel: dalla 3^ linea in poi
  - Liso-cel: grado 3B dalla III linea in poi (18-75 anni)

ELIANA: Tisagenlecleucel in Children and Young Adults With R/R B-ALL

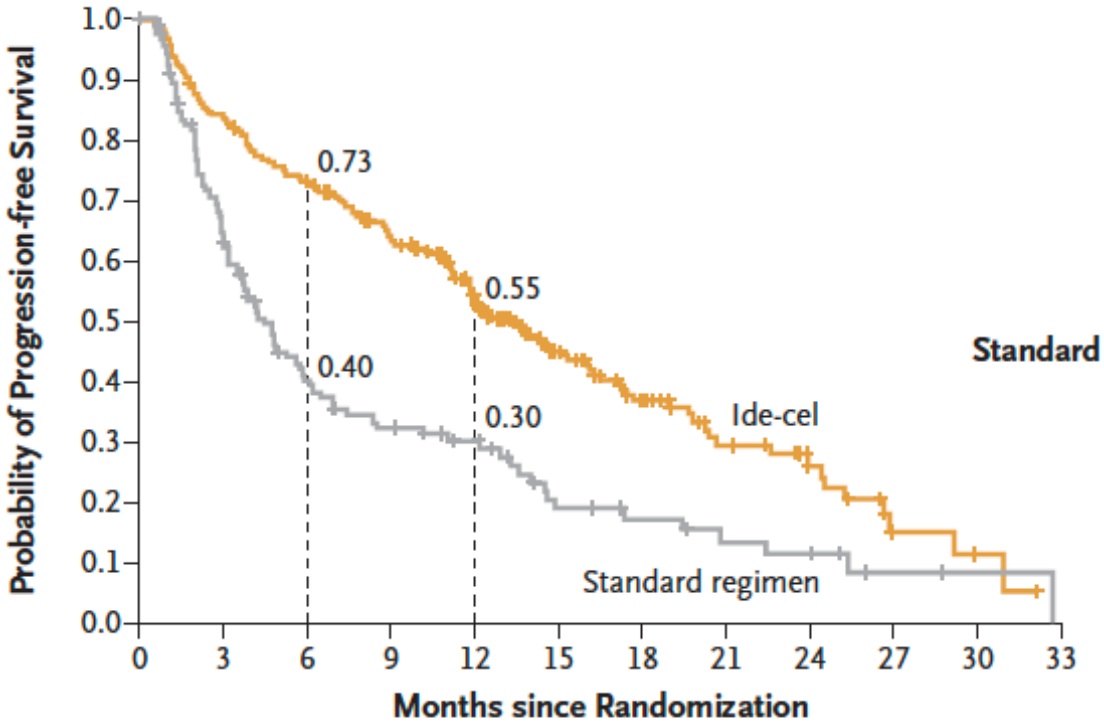
- International, open-label, single-arm phase II study (N = 92)
  - Patients aged 3-21 yrs with relapsed or refractory B-cell ALL
  - Patients underwent lymphodepletion with fludarabine + cyclophosphamide followed by single-dose tisagenlecleucel
  - At baseline: median number of prior therapies, 3; prior allogeneic SCT, 46%; median BM blast count at time of treatment, 74%
- ORR at 3 mos: 81%

Outcome, %	Mo 6	Mo 12
OS	90	76
Event-free survival	73	50



Maude. NEJM. 2018;378:439.

KarMMa: Idecabtagene Vicleucel for R/R Multiple Myeloma



No. at Risk

Ide-cel	254	206	178	149	110	62	40	22	14	4	2	0
Standard regimen	132	75	42	32	25	13	10	7	6	2	1	0

	Median Progression-free Survival (95% CI)
	mo
Ide-cel	13.3 (11.8–16.1)
Standard Regimen	4.4 (3.4–5.9)

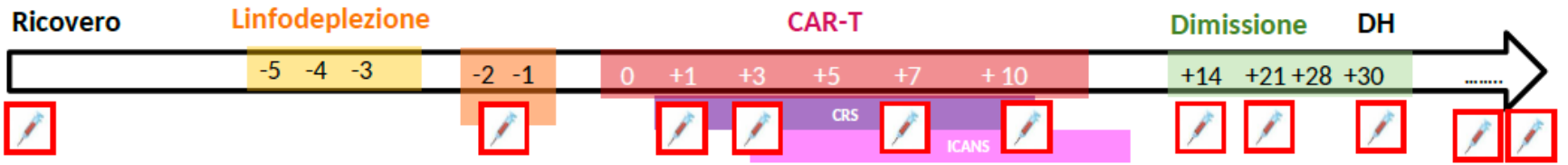
Hazard ratio for disease progression or death, 0.49 (95% CI, 0.38–0.65)
P<0.001

Rodriguez-Otero et al., NEJM 2023

CAR-T
Gestione delle complicanze

1. CRS (Cytokine Release Syndrome)
2. ICANS (Immune effector Cell-associated Neurotoxicity Syndrome)
3. HLH/MAS (Hemophagocytic Lymphohistiocytosis Macrophage Activation Syndrome)

Bio CAR-T BS study Flow Chart



Monitoraggio medico

- Scheda Protocollo di ingresso
- Disponibilità Tocilizumab x 4
- Avviso generale al CAR-T team

- Valutazione Tossicità chemioterapia

- Test scrittura da -1
- Contattare Neurologi, Intensivisti, Radiologi, Infettivologi

- Valutazione insorgenza CRS e ICANS,

Se CRS:
-Emocolture/urocoltura→ inizio tp antibiotica secondo BATMO
-Contattate RIA

Se ICANS:
-Contattare Neurologo, EEG, RM cerebrale
-Valutare PL e fundus oculi

- Valutare Neutropenia→considerare G-CSF
- Valutare Ipogammaglobulinemia→considerare Ig vena

- Valutazione insorgenza CRS e ICANS: questionario telefonico x1/g

Monitoraggio clinico-infermieristico

- Verifica presidi di aspirazione

- PV da routine clinica

- ICE score x2/g
- Valutazione stato coscienza, crisi epilettiche, anomalie motorie, cefalea, tremori, comportamento

Se CRS:
-ogni 4 h: PA, Sat, FR, FC, T°, stato coscienza, vigilanza, dolore
-ogni 8 h: Diuresi
-Esami secondo "Schema esami degenza CAR T"

Studi biologici

Al ricovero:
- CAR-T real life (3 provette EDTA 4.9 mL)
- Citochine; cfDNA; Cellule vitali
- Sottopopolazioni Ly
- Microbiota
- Prot. Vescicole (2 EDTA 4.9 mL, Simona)
- Immunofenotipo (1 EDTA 4.9 mL Chiarini)

-2 o -1:
- CAR-T real life
- Citochine; cfDNA; Cellule

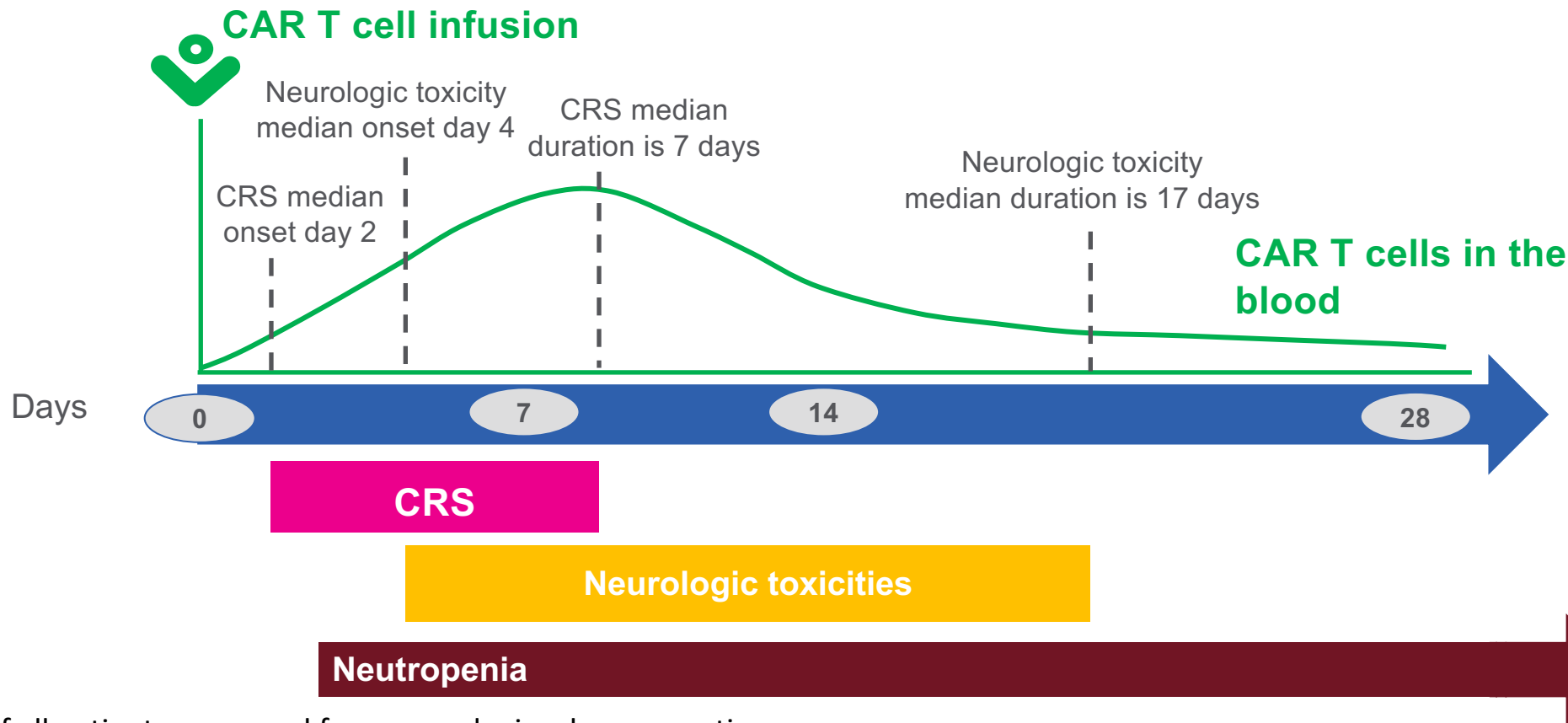
+1 e +3:
- CAR-T real life
- Citochine; cfDNA; Cellule
- Prot. Vescicole
- dPCR (Lanfranchi)
- Immunofenotipo

+7; +10; +14; +21; +30, mensilmente fino all'anno o alla recidiva e allo sviluppo di CRS/0neurotossicità:
- Immunofenotipo *(invio a Milano o localmente)*
- CAR-T real life (Citochine; cfDNA; Cellule)
- Prot. Vescicole
- dPCR (Lanfranchi)

+30; +6mesi; +1aa o alla recidiva:
- Microbiota

+10; 30; +90; +180; +1aa o alla recidiva:
- Sottopopolazioni Ly

Timing of toxicities



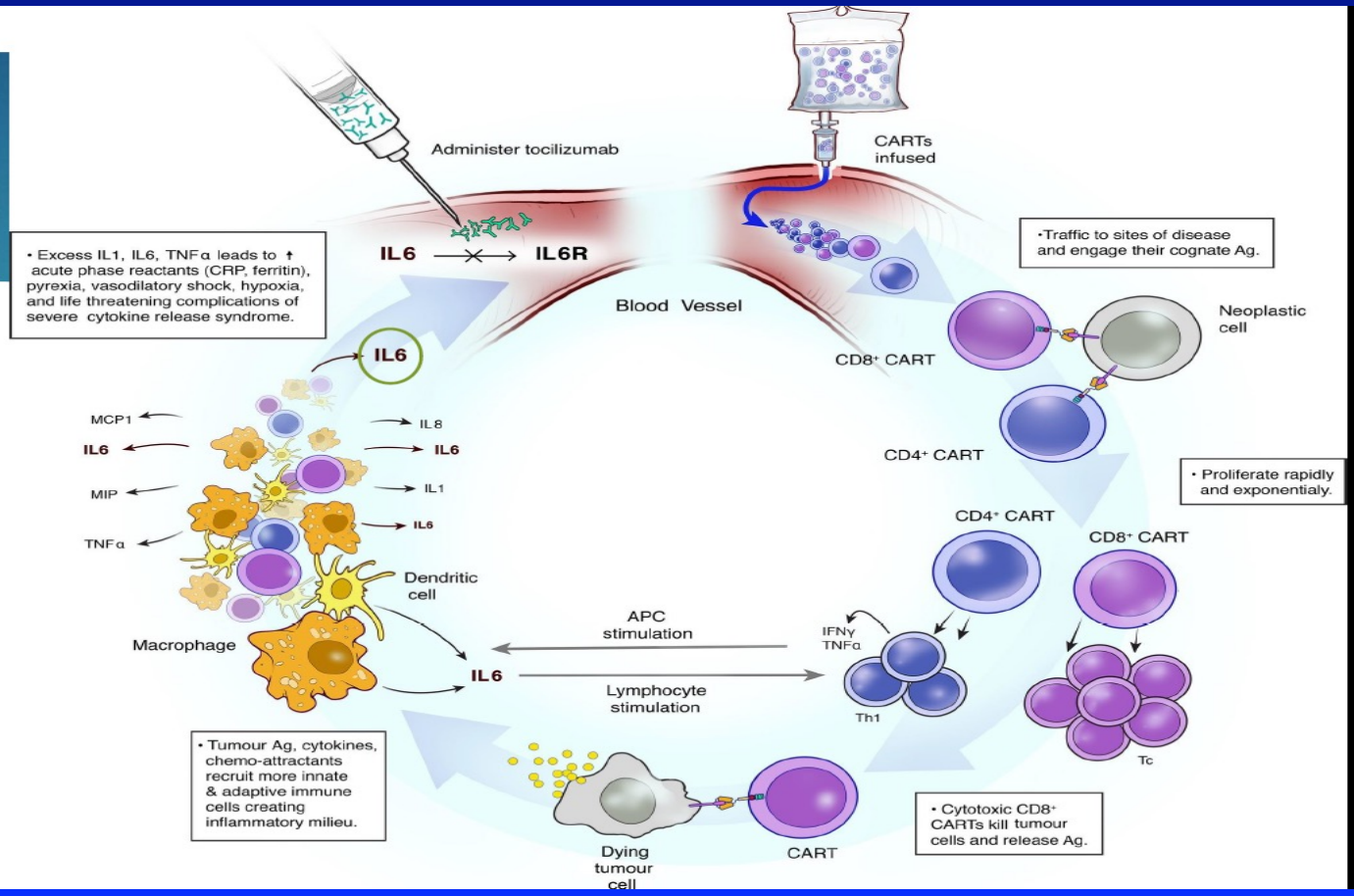
98% of all patients recovered from neurologic adverse reactions

98% of all patients recovered from CRS

Adapted from Lee DW, et al. *Blood*. 2014;124:188-195. 2.

CRS


7:57
Cancer
CARTOX
Toxicity Assessment and Management
Toxicity Grading
Toxicity Management
Antimicrobial Prophylaxis
About Us

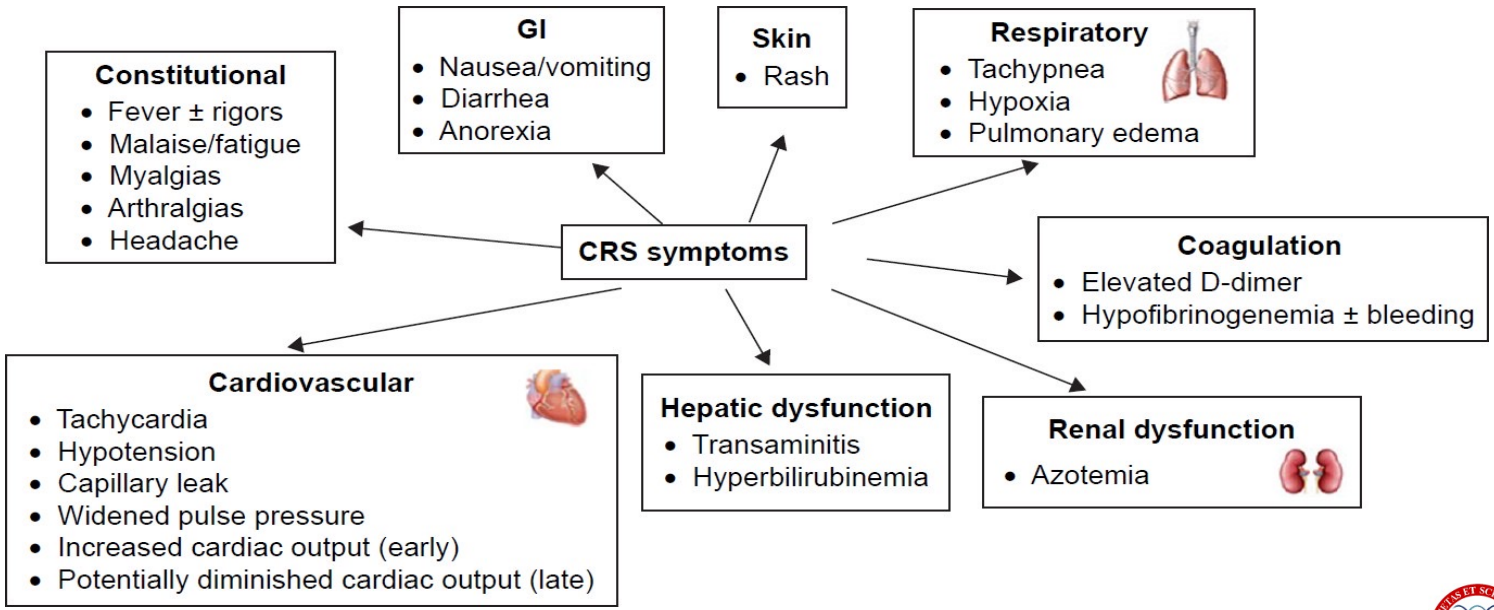

Administer tocilizumab
CARTs infused
IL6
IL6R
• Excess IL1, IL6, TNFα leads to ↑ acute phase reactants (CRP, ferritin), pyrexia, vasodilatory shock, hypoxia, and life threatening complications of severe cytokine release syndrome.
Blood Vessel
•Traffic to sites of disease and engage their cognate Ag.
Neoplastic cell
CD8⁺ CART
CD4⁺ CART
• Proliferate rapidly and exponentialy.
IL6
MCP1
IL8
IL6
IL6
MIP
IL1
IL6
TNFα
Dendritic cell
Macrophage
APC stimulation
IL6
Lymphocyte stimulation
IFNγ TNFα
Th1
Tc
• Tumour Ag, cytokines, chemo-attractants recruit more innate & adaptive immune cells creating inflammatory milieu.
Dying tumour cell
CART
• Cytotoxic CD8⁺ CARTs kill tumour cells and release Ag.

Cytokine release syndrome (CRS)

CLINICAL SYMPTOMS

From mild flu-like symptoms → Severe inflammatory syndrome

CLINICAL EFFECTS



Riegler and al., Therapeutics and clinical risk managment, 2019

Factors associated with risk of CRS

CAR product

- Costimulatory domain: CD28 vs 4-1BB
- CD4/CD8 ratio
- Cell dose
- Peak of in vivo expansion

Other factors

- Lymphodepletion regimen
- Early onset CRS (< 2 days)

Patient and Disease

- High tumor burden before LD (i.e BM involvement; TMTV; LDH)
- Aggressive disease vs Indolent
- Endhotelian Activation signs (elevated CRP, Thrombocytopenia)
- mEASIX score = (lactate dehydrogenase [LDH in U/L] x C-reactive protein [CRP in mg/dL]/platelets [PLTs in 109 cells/L])

Lee DW, et al. *Lancet. 2015;385:517–528.*
Neelapu SS, et al. *Nat Rev Clin Oncol. 2018;15:47–62.*
Hay KA, et al. *Blood. 2017;130(21):2295-2306.*

CAR-T toxicities in Clinical trials

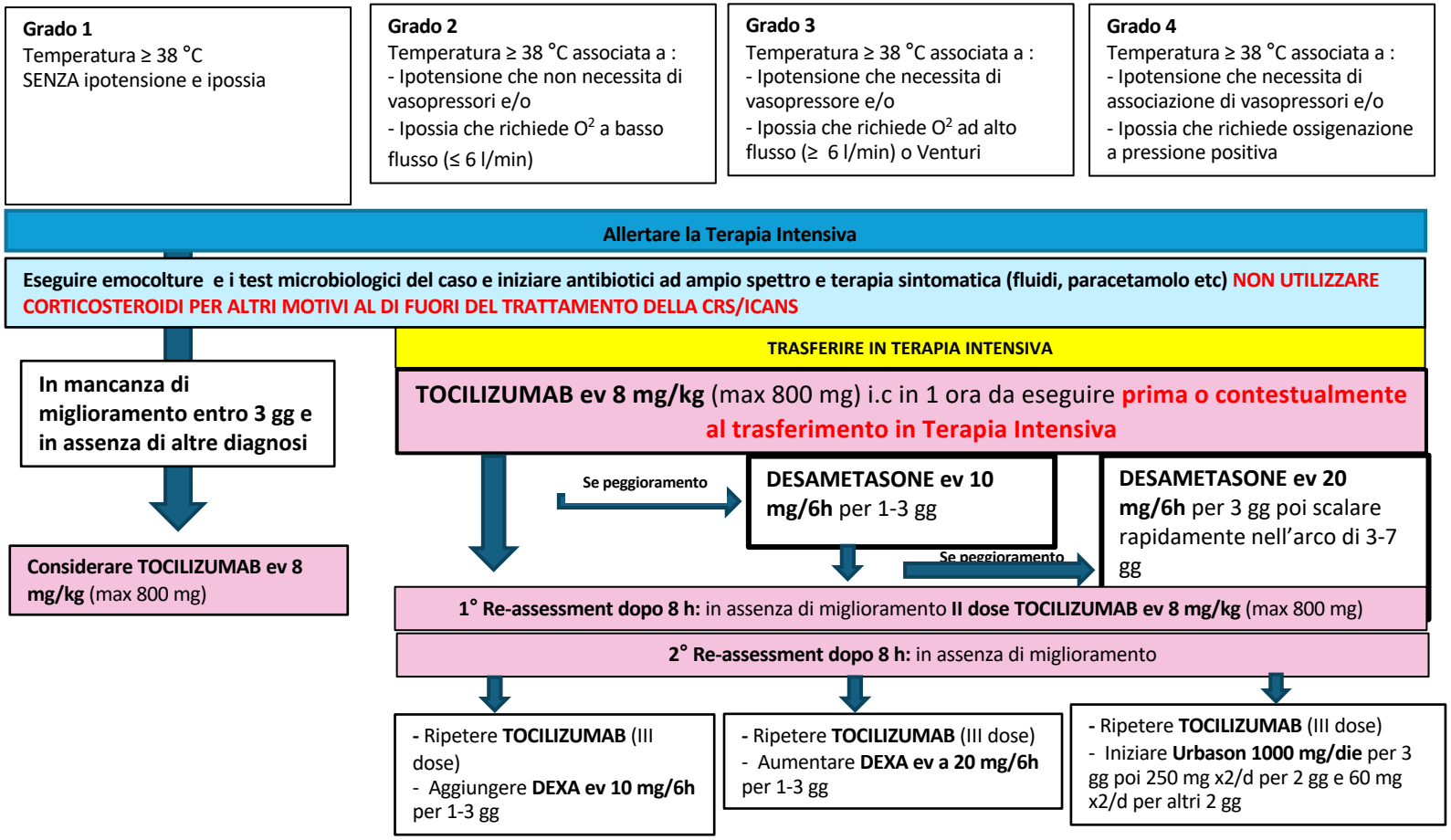
Product	CRS			ICANS/Neurologic Events			Other AEs
	Incidence: Any Grade/ Grade ≥3, %	Median Time to Onset, Days	Median Duration, Days	Incidence: Any Grade/ Grade ≥3, %	Median Time to Onset, Days	Median Duration, Days	
Axicabtagene ciloleucel[8,10-12]	82-93/6-13	2-4	6-7	59-64/19-28	4-6	15-17	• Prolonged grade ≥3 cytopenias: 29% to 34% • Hypogammaglobulinemia: 11% to 18% • B-cell aplasia at 12 months: 36% • Grade ≥3 infections: 14% to 18%
Brexucabtagene autoleucel[14,19]	91-92/18-26	3-5	8-10	81-87/35-37	6-7	15-21	• Prolonged grade ≥3 cytopenias: 36% to 55% • Hypogammaglobulinemia: 9% to 16% • Grade ≥3 infections: 25% to 32%
Ciltacabtagene autoleucel[22]	95/5	7	4	23/3	8	7.5	• Prolonged grade ≥3 neutropenia: 30% • Hypogammaglobulinemia: 12% • Grade ≥3 infections: 23%
Idecabtagene vicleucel[21]	85/9	1	7	28/4	2	6	• Prolonged grade ≥3 neutropenia: 41% • Hypogammaglobulinemia: 21% • Grade ≥3 infections: 23%
Lisocabtagene maraleucel[20]	36-49/1-2	4-5	4-5	12-31/4-10	7-11	6-11	• Prolonged grade ≥3 cytopenias: 30% to 43% • Hypogammaglobulinemia: 8% to 14% • B-cell aplasia at 2 mo: 97% • Grade ≥3 infections: 7% to 15%
Tisagenlecleucel[9]	53-77/0-48	3-4	4-8	43-71/6-22	5-8	5-17	• Prolonged grade ≥3 neutropenia: 16% to 40% • Hypogammaglobulinemia: 17% to 53% • Grade ≥3 infections: 21% to 48%

ASTCT Guidelines for Grading of CRS

Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever	Temp ≥ 38°C	Temp ≥ 38°C	Temp ≥ 38°C	Temp ≥ 38°C
with				
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
and/or				
Hypoxia	None	Requiring low-flow nasal cannula or blow-by	Requiring high-flow nasal cannula, facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (eg, CPAP, BiPAP, intubation, and mechanical ventilation)

Lee. Biol Blood Marrow Transplant. 2019;25:625.

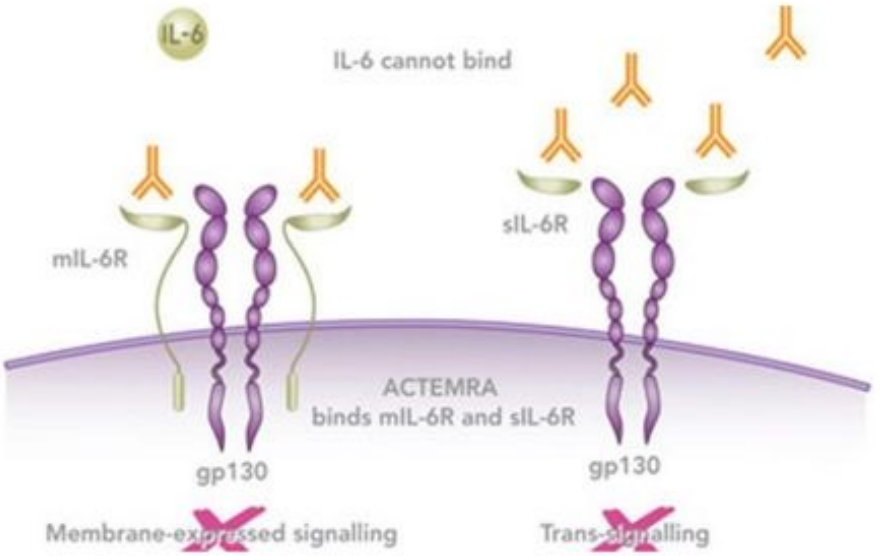
CRS management according ASCTC



Lee. Biol Blood Marrow Transplant. 2019;25:625.

Tocilizumab

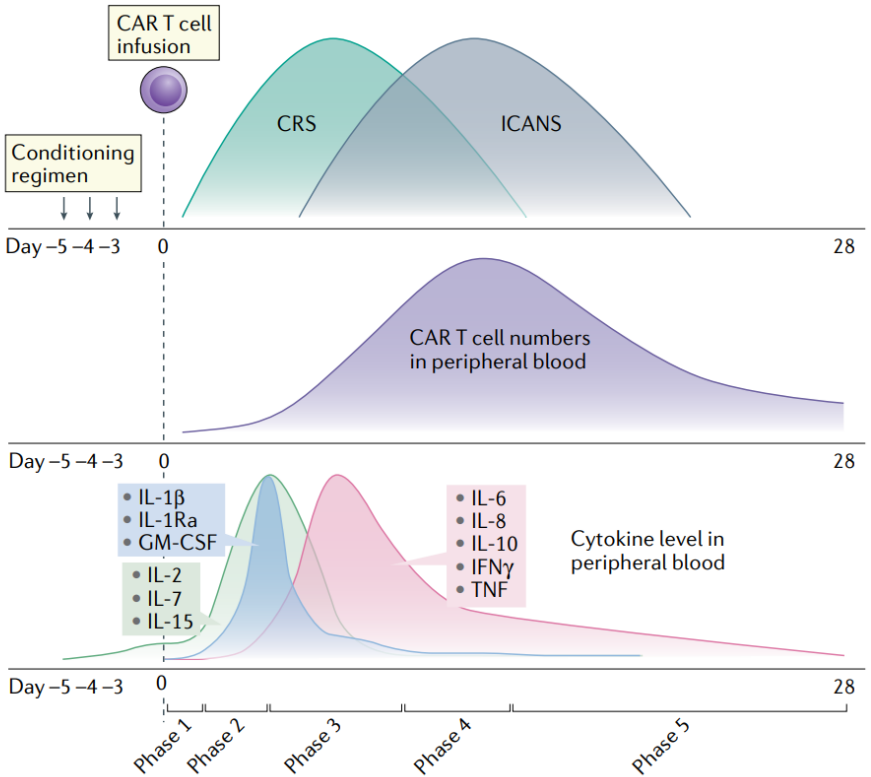
- Humanized anti-IL-6 receptor monoclonal antibody
- Binds directly to IL-6 receptor→ blocks binding and signaling
- FDA approval for CAR-T-cell induced CRS
- Dosing: 8 mg/kg IV Q6H for up to 3 doses in a 24-hour period (max of 4 total doses)
- Median time to defervescence is 4 hours



Neelapu et al., Nat Rev Clin Oncol 2018

Neurotossicità

ICANS= Immune effectors Cells Associated Neurologic Syndrome



- Attivazione endoteliale
- Permeabilità microvascolare
- Alterazione della barriera emato-encefalica
- Increased level of cytokines:

  IL1a, IL6, IL10, G-CSF, TNFα, IFNγ, IFNα2, FLT3L,

  eotaxin, fractalkine, and GRO

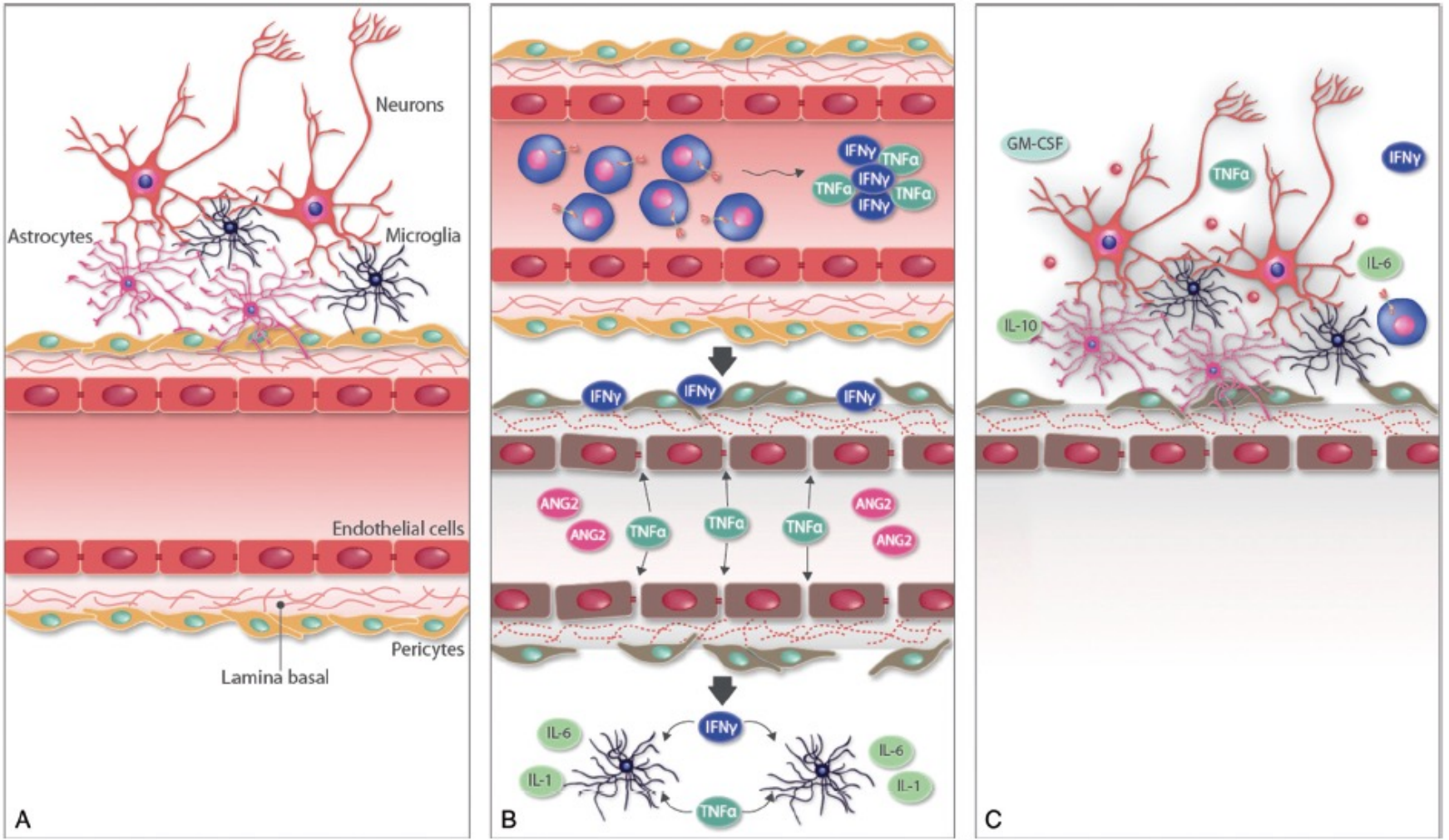
Morris et *al.*,*Nature rev/immunology 2022*

Santomasso et *al.*, cancer discovery, 2018

BEE NORMALE

CAR-T cells: ↑ TNF α e INFy

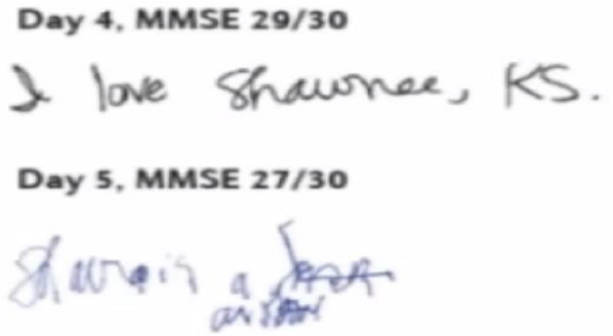
↑ citochin (IL6, IL10; TNF α e INFy)
--> attivazione microglia



Yáñez et al., HemaSphere 2019

Immune effector cell-associated neutotoxicity syndrome (ICANS)

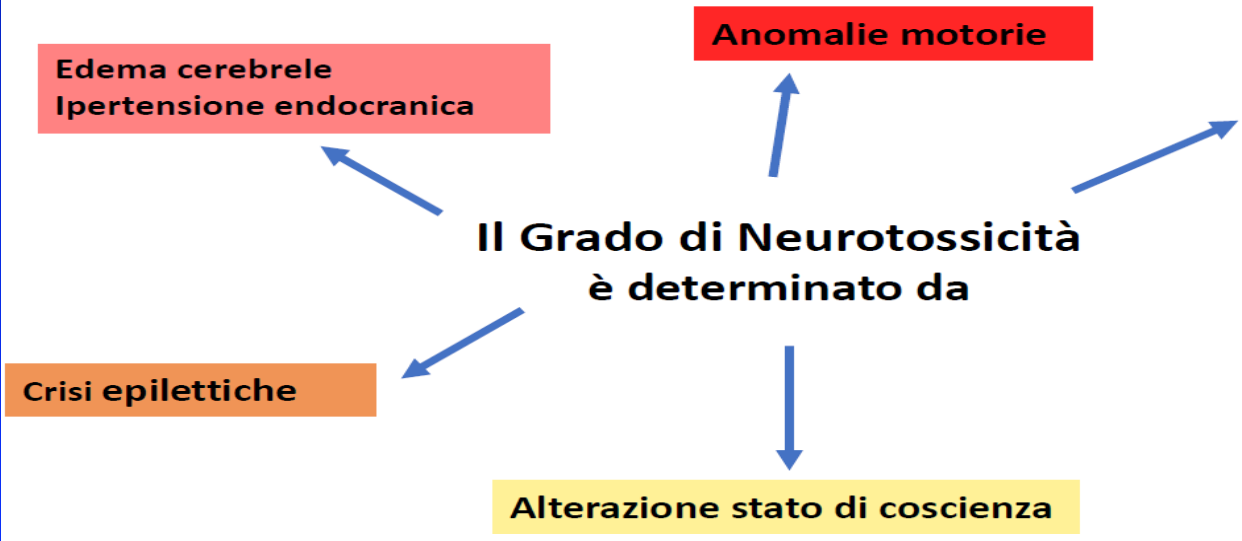
- **Encefalopatia** su base tossica, con i seguenti sintomi:
  - AFASIA espressiva
  - Deterioramento della scrittura
  - Moderata **cefalea**
  - Sonnolenza
  - Tremori
  - **Confusione e delirum**
  - **Crisi epilettiche**
  - **Edema cerebrale**
  - **Incontinenza**



- **Esami strumentali:**
  - **EEG:** rallentamento diffuso o convulsioni
  - **Liquor** positivo per CARTs
  - **RM**: edema in casi più gravi

2) ICANS - CRITERI PRINCIPALI della Tossicità Neurologica

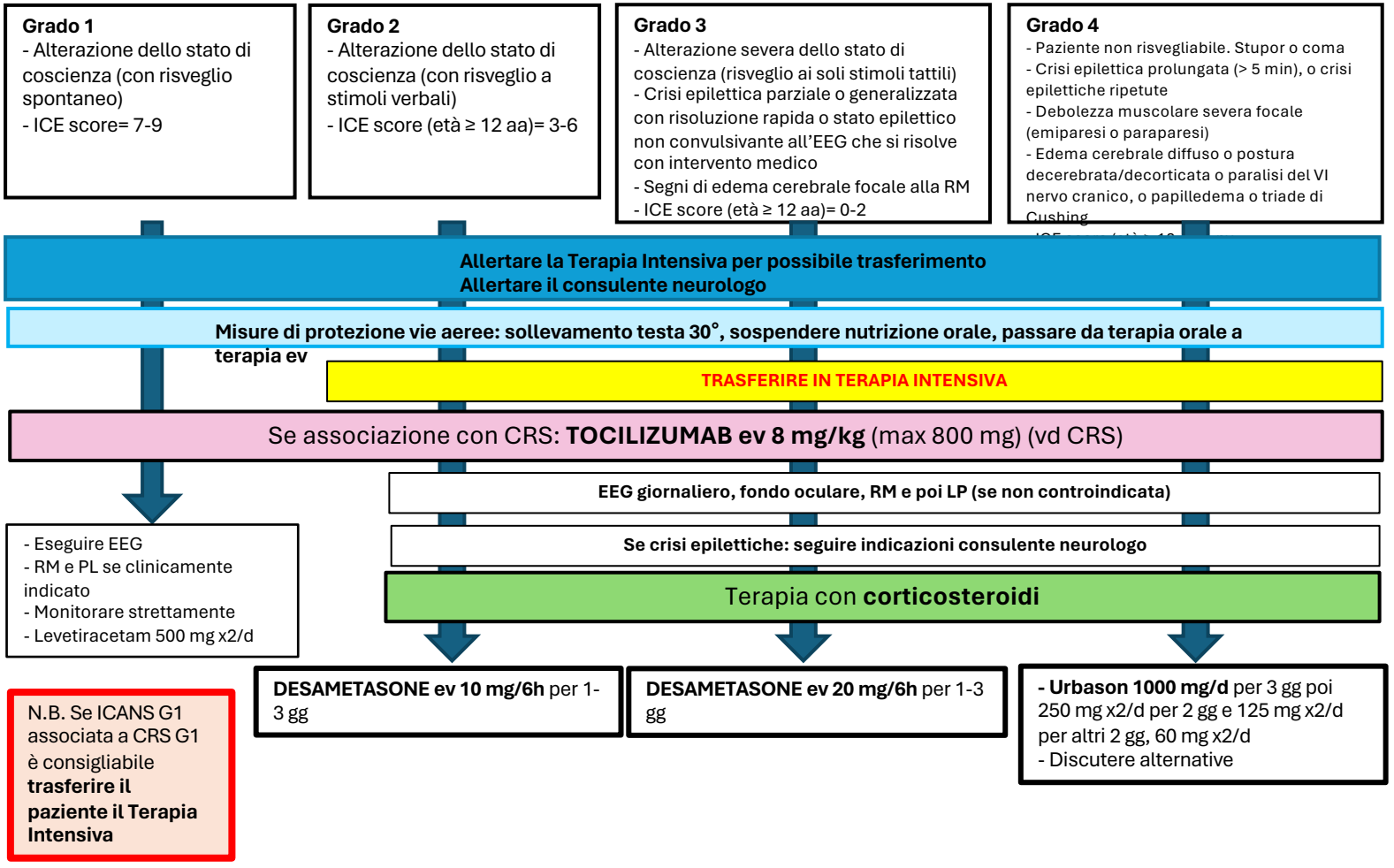
Ogni sintomo neurologico deve essere considerato CAR T correlato fino a prova contraria



ICE (Immune effector Cell-associated Encephalophaty):

- ORIENTAMENTO- il pz è in grado di dichiarare anno, mese, città, ospedale (*4 punti*)
- DENOMINAZIONE- il pz è in grado di nominare 3 oggetti (*3 punti*)
- ESECUZIONE COMANDI MOTORI – il pz è in grado di eseguire 3 comandi motori semplici (*1 punto*)
- SCRITTURA- scrivere una frase semplice (1 punto)
- ATTENZIONE- contare all'indietro di 10 in 10 a partire da 100 (*1 punto*)

Grading Neurotossicità (ICANS)



3) HLH/MAS

CRITERI DIAGNOSTICI

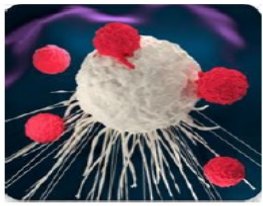
1. CRS

2. Ferritina> 10.000ng/ml

Almeno 2 tra:

- ≥ **Iperbilirubinemia** (x3-10v ULN) o incremento AST e/o ALT (x 5-20v ULN)
- **Oliguria** (<80ml in 8h) o **incremento creatininemia** (x3v baseline o x3-6v ULN)
- **Edema polmonare** (dispnea a riposo, fabbisogno O2)
- **Emofagocitosi confermata** da reperti morfologici e CD68+ al BM/organi

GESTIONE di sospetta HLH/MAS



TOCILIZUMAB + CORTICOSTEROIDI
a seconda del grado CRS

↓

Monitorare ferritina, LDH, fibrinogeno, AST, ALT, GGT, bilirubina, creatinina

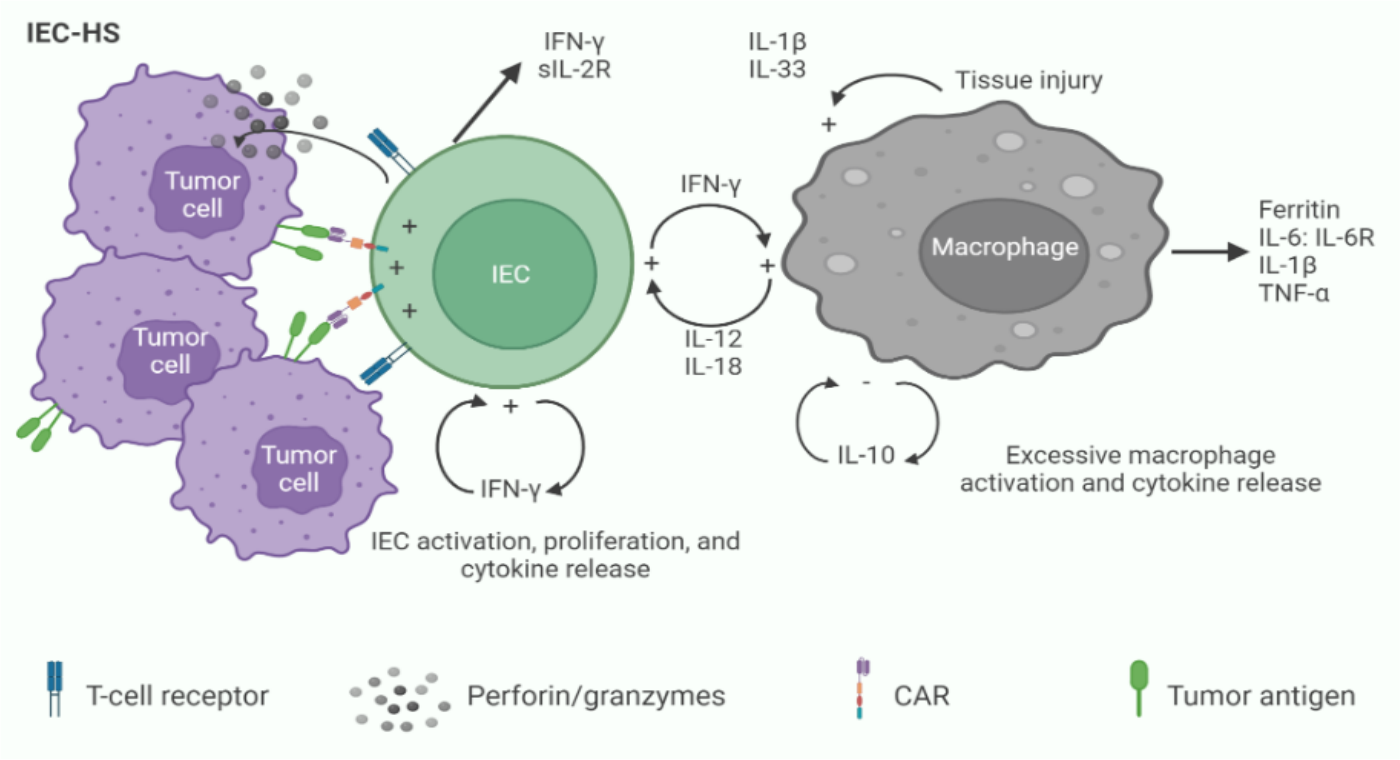
↓

Dopo 48h, miglioramento?

- SÌ → Prosegui come per CRS
- NO → Considera
  ETOPOSIDE 75-100mg/m2 ogni 4-7gg
  CITARABINA intratecale 100mg +/-
  IDROCORTISONE 50-100mg per neurotossicità

Immune Effector Cell-Associated Hemophagocytic Lymphohistiocytosis-Like Syndrome (IEC-HS)

Underlying mechanism of pathologic immune activation in IEC-HS*



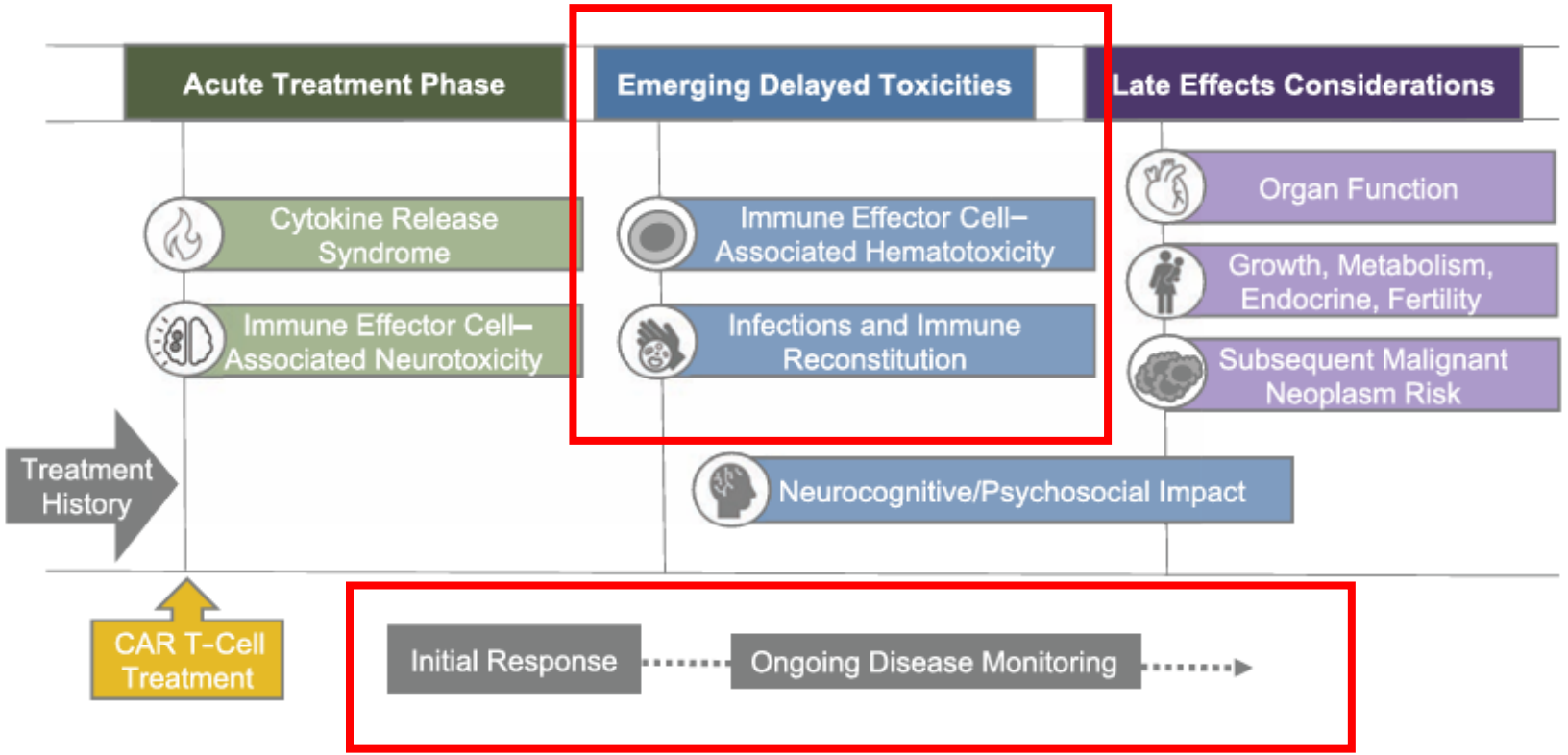
Prevalenza: <5%

Mortalità: >50%

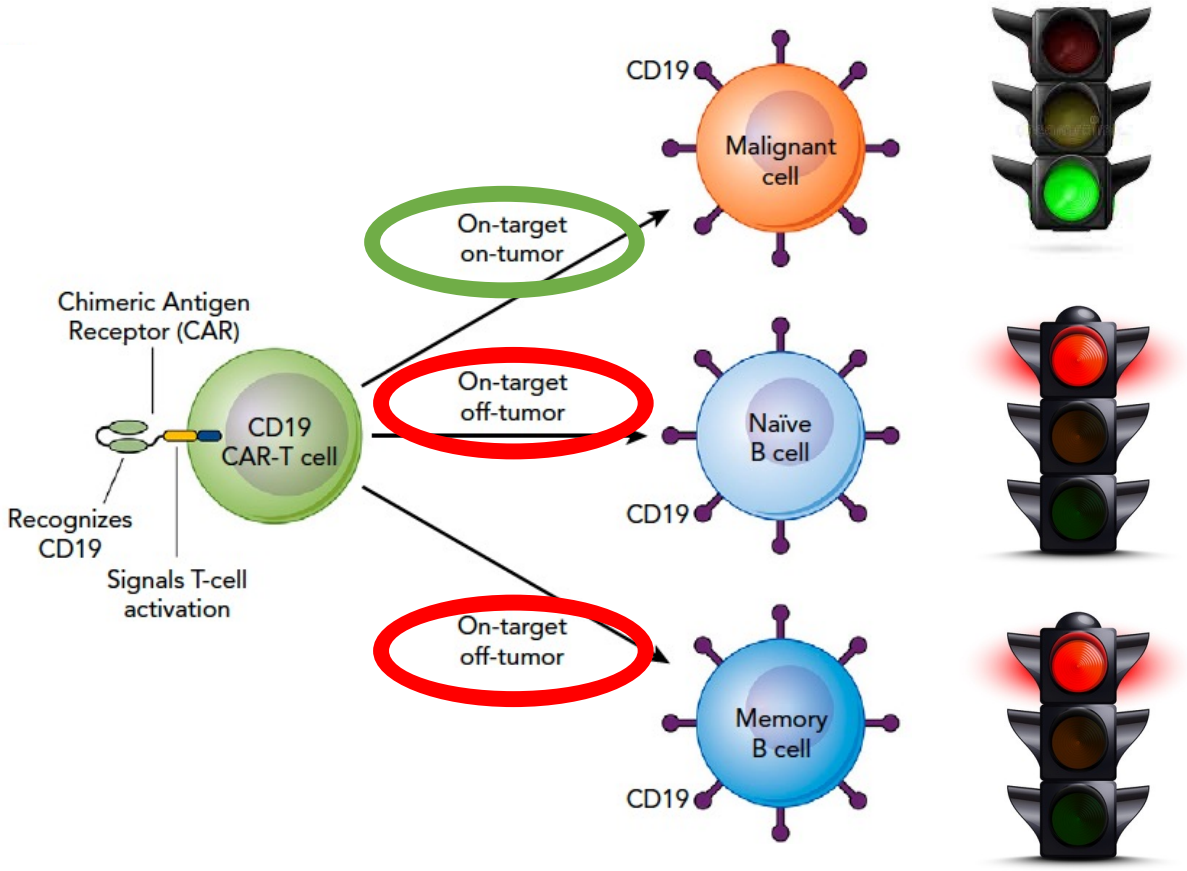
CAR, chimeric antigen receptor; IEC, immune effector cell; IEC-HC, IEC-associated HLH-like syndrome IFN-γ, interferon gamma; IL, interleukin; R, receptor; sIL-2R, high soluble interleukin 2-R; TNF-α, tumor necrosis factor α.

Hines MR, Knight TE, et al. Immune Effector Cell-Associated Hemophagocytic Lymphohistiocytosis-Like Syndrome. Transplant Cell Ther. 2023

Long-term follow-up of CD19-CAR Tcell therapy



Epperly et al. Hematology 2023 | ASH Education Program


Chimeric Antigen Receptor (CAR)
CD19 CAR-T cell
Recognizes CD19
Signals T-cell activation
On-target on-tumor
On-target off-tumor
On-target off-tumor
CD19
Malignant cell
Naïve B cell
CD19
Memory B cell
CD19

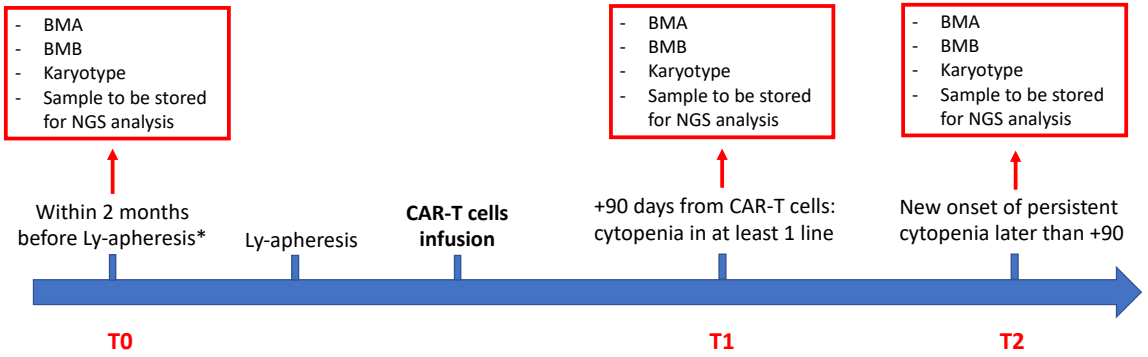
ClonHema-CAR-T Study

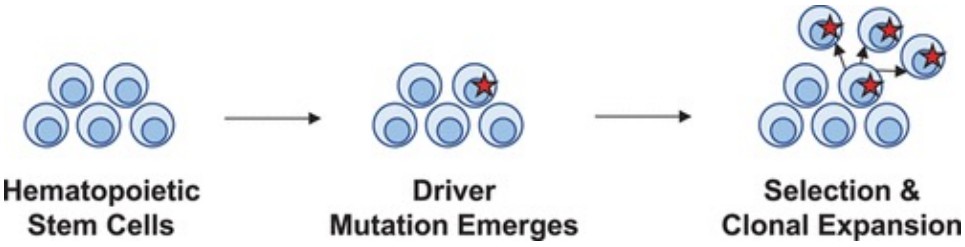
Study of clonal hematopoiesis on patients undergoing CAR-T cells therapy

(ClonHema-CAR-T Study)

PI: Prof. D. Russo

✓ **Approved**



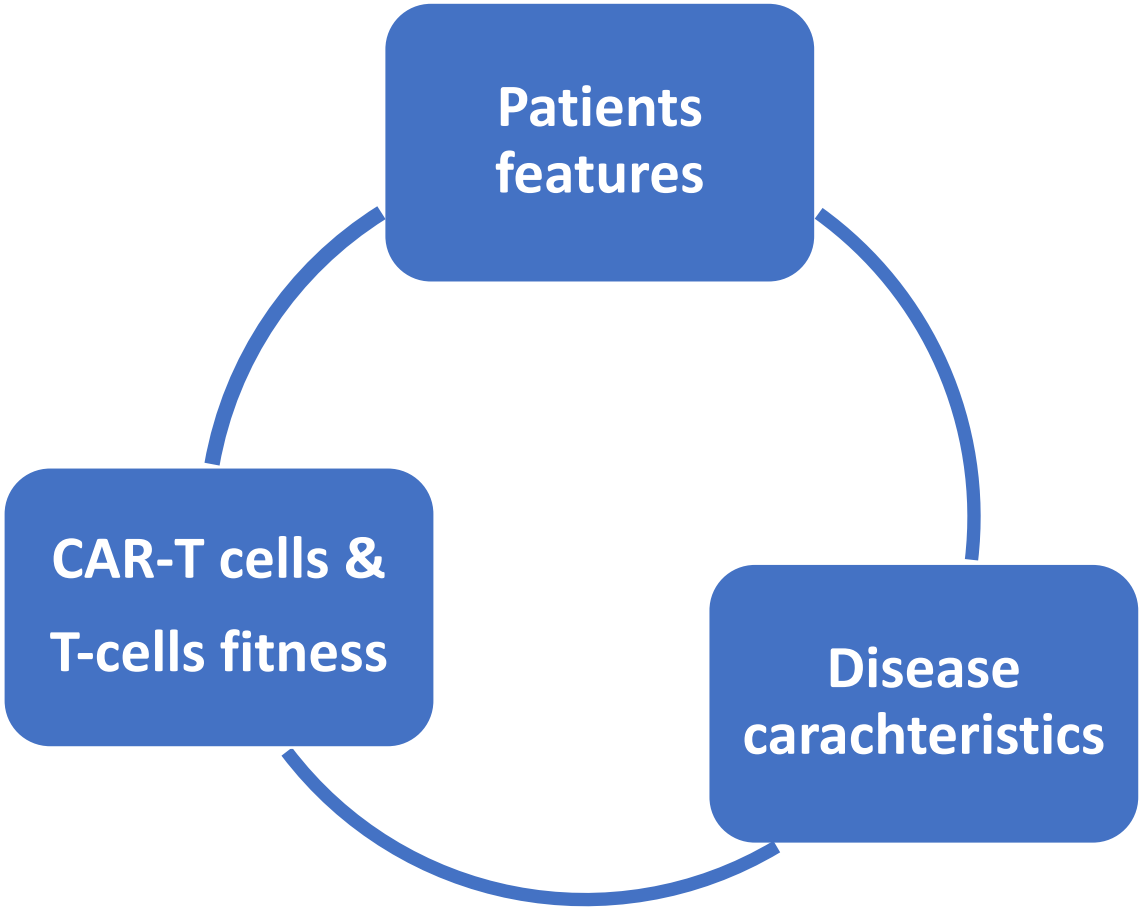


GITMO

GRUPPO ITALIANO PER IL TRAPIANTO DI MIDOLLO OSSEO, CELLULE STAMINALI EMOPOIETICHE E TERAPIA CELLULARE

- *Bergamo – A. Rambaldi*
- *Brescia – D. Russo*
- *Firenze - R. Saccardi*
- *Milano (Humanitas Cancer Center) – S. Bramanti*
- *Milano (San Raffaele) – F. Ciceri*
- *Milano (Niguarda) – G. Grillo*
  Napoli – F. Pane
- *Padova – A. Biffi*
- *Palermo – M. Musso*
- *Pescara – M. Di Ianni*
- *Reggio Calabria – M. Martino*
- *Roma (Gemelli) – S. Sica*
- *Roma (Umberto I) – A.P. Iori*
- *Vicenza – C. Borghero*

Factors associated with the response to CAR-T



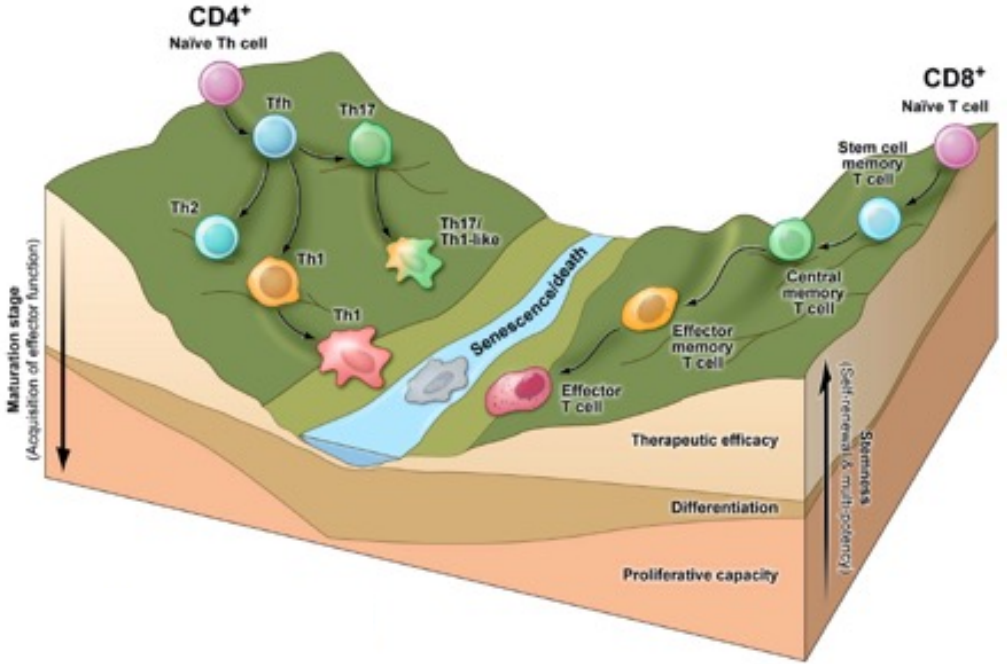
T-cells fitness

Not all T cells are created equally

CD45RA	+	+	−	−	+
CD45RO	−	−	+	+	−
CCR7	+	+	+	−	−
CD62L	+	+	+	−	−
CD28	+	+	+	+/−	−
CD27	+	+	+	+/−	−
IL-7Rα	+	+	+	+/−	−
CXCR3	−	+	+	−	−
CD95	−	+	+	+	+
CD11a	−	+	+	+	+
IL-2Rβ	−	+	+	+	+
CD58	−	+	+	+	+
CD57	−	−	−	+/−	+

Stemness	Senescence
Proliferative potential	Cytotoxicity
Lymphoid homing	Tissue tropism
Antigen independence	Antigen addiction
Lipid metabolism	Glycolytic metabolism
Low ΔΨm	Oxidative stress

Impairment of antitumor efficacy with progressive T cell differentiation

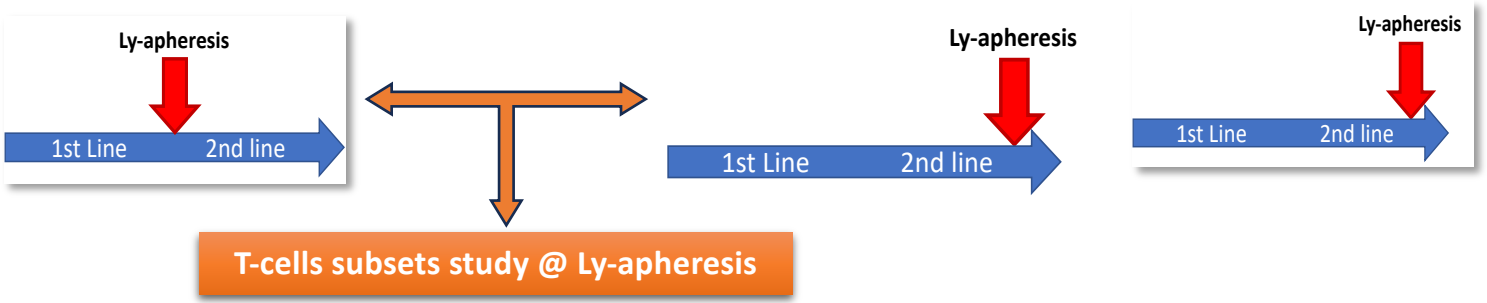


-CAR-T BS Study: Pre-emptive/early Ly-apheresis program

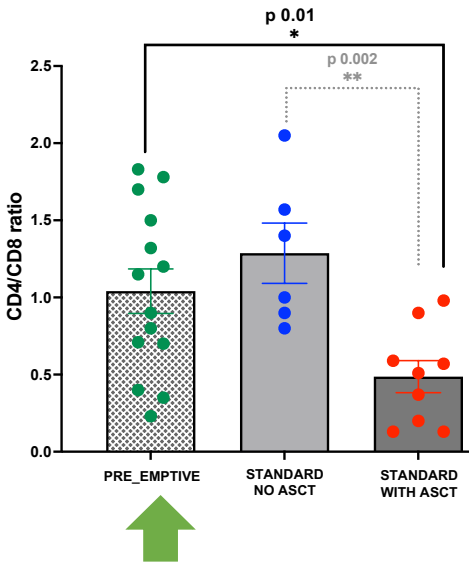
«PRE-EMPTIVE Ly-apheresis»

HIGH RISK DLBCL patients

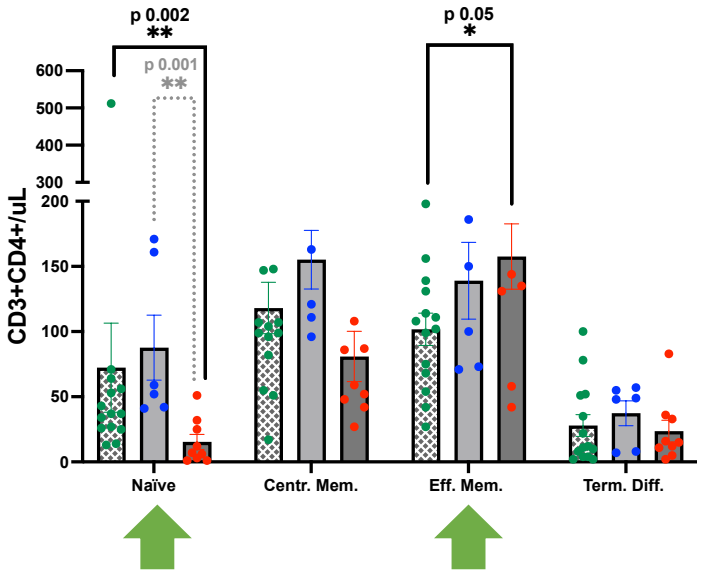
- *PRIMARY REFRACTORY*
- *PET+ before ASCT*
- *RELAPSE WITHIN 1 year*



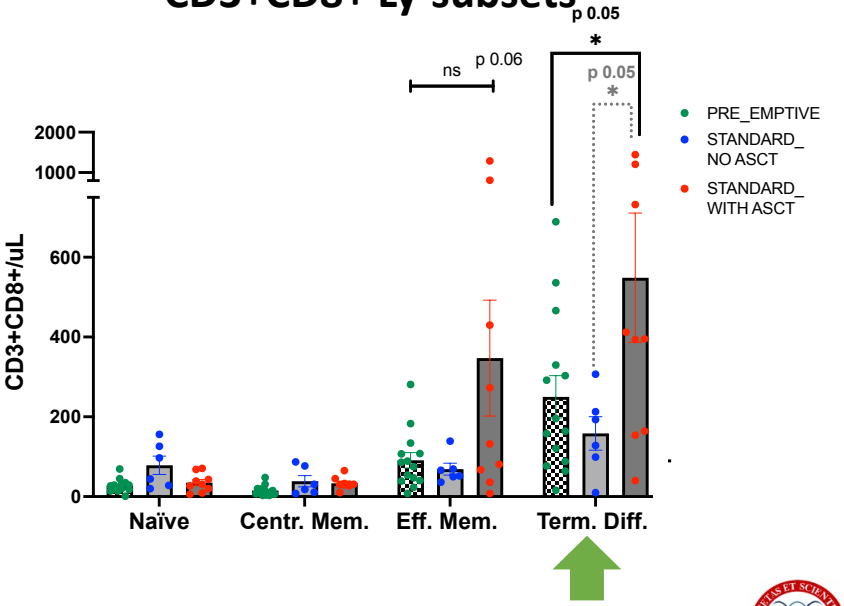
CD4+/CD8+ ratio



CD3+CD4+ Ly-subsets



CD3+CD8+ Ly-subsets

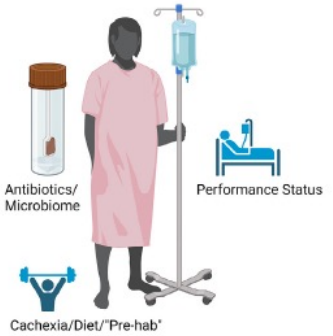


Farina et al., Cancers 2022

Strategies to optimize CAR-T cells therapy

Patients features

AVOID DETERIORATION of HOST FACTORS

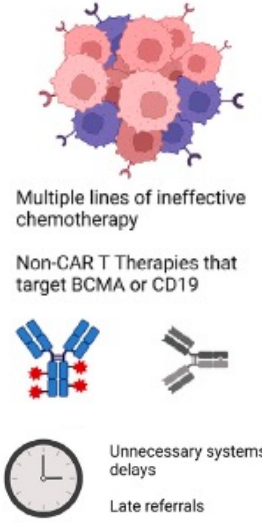


CAR-T cells product

IMPROVE T cells FITNESS OPTIMIZE CAR-T cells

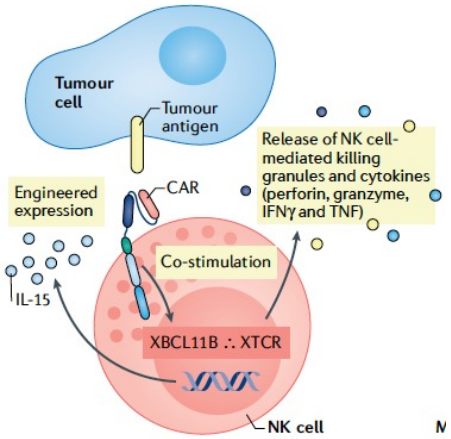
Disease characteristics

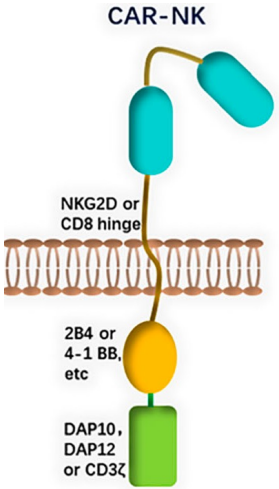
AVOID TUMOR-INTRINSIC RESISTANCE

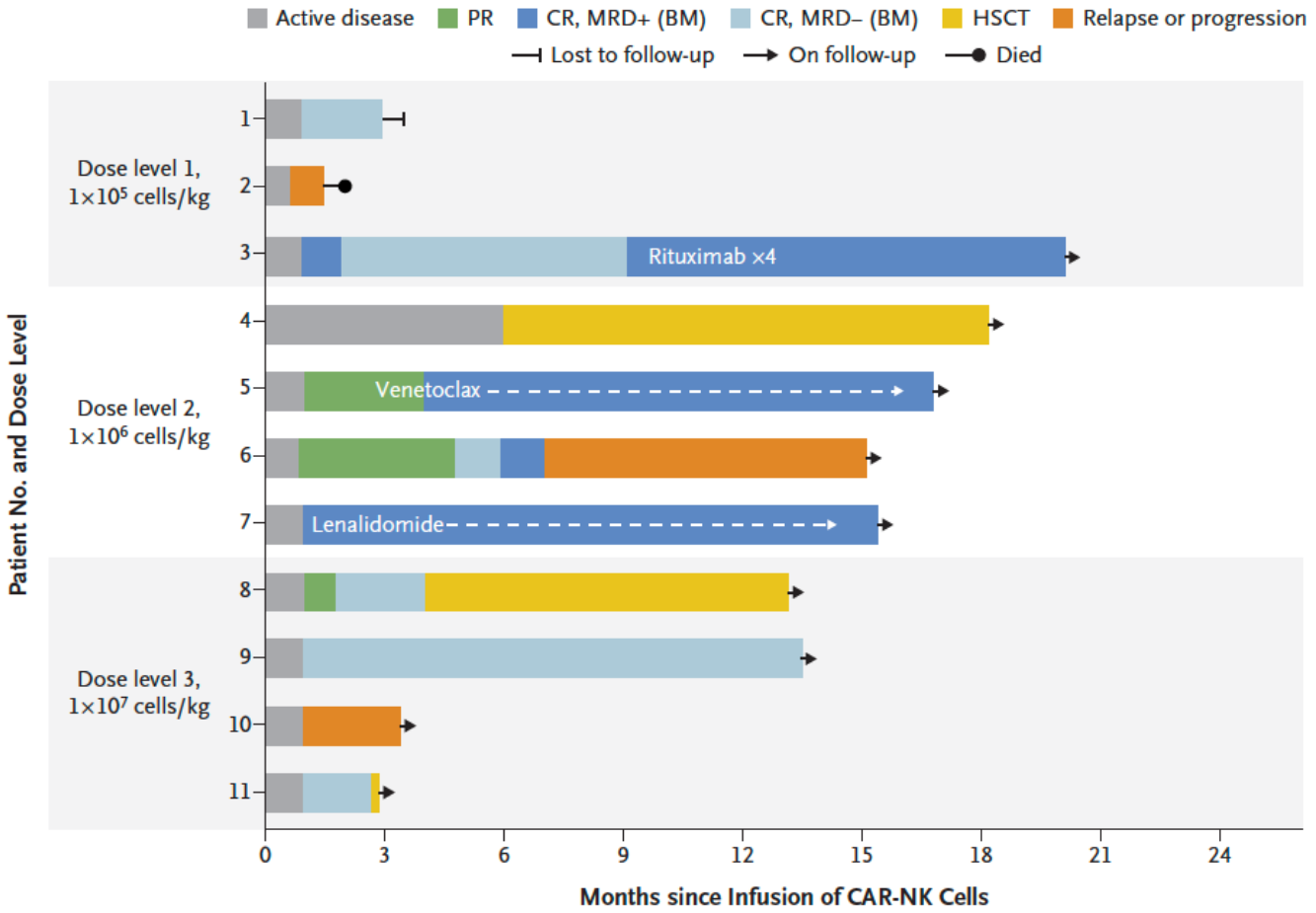


Rejeski et al. Transpl and Cell Ther 2023

NK cells as alternative source of CAR-T

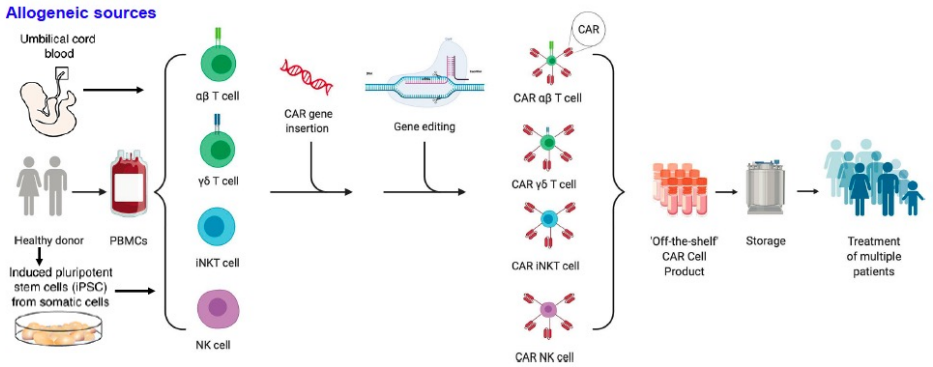






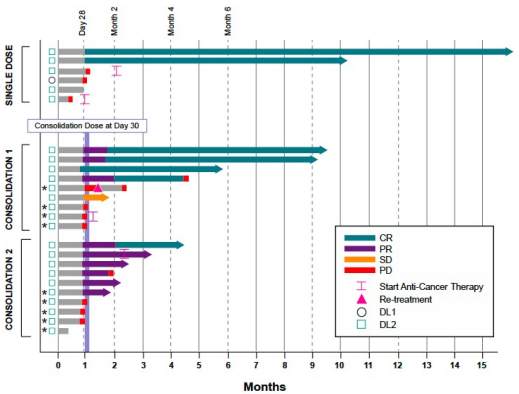
Sivori et al., J. Clin. Med. 2019; Liu et al., NEJM 2020

Efficacy in phase 1 allogeneic CAR-T trials in r/r DLBCL



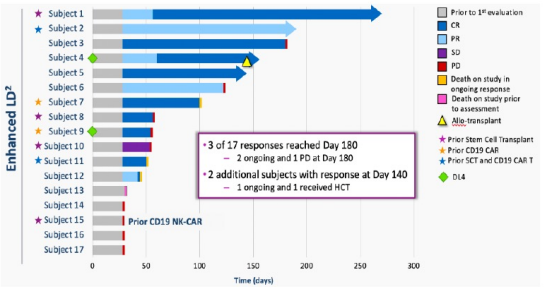
ALLO-501 (CD19 αβ CAR)

ORR/CR rate % = 48/28



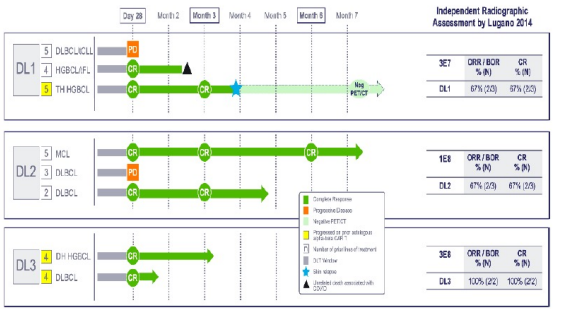
PBCAR0191 (CD19 αβ CAR)

ORR/CR rate % = 69/56



ADI-001 (CD20 γδ CAR)

ORR/CR rate % = 75/75



- No GVHD, Grade ≥3 NE or CRS

- ↑ grade ≥3 infections

Lekakis et al., ASH 2021 Abs 649; Shah et al., ASH2021 Abs 302; Neelapu et al. ASCO 2022, Abs 7509