TC-A per DLI:
Indicazioni e controlli di qualità

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DLI: storia

1990: *Kolb infuse linfociti del donatore in paziente ricaduto di LMC inducendo remissione citogenetica*

DLI = donor lymphocyte infusion

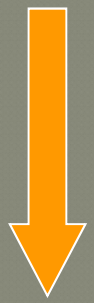
Il trapianto di cellule staminali allogeniche è stata la prima forma di tearapia cellulare applicata su larga scala

- L'effetto antileucemico insito nel graft ha chiarito e incrementato le strategie per ridurre recidive

- La deplezione di cellule T dal graft riduce la possibilità di GvHD
  Aumenta la possibilità di recidiva di malattia e di graft failure

DLI

Cellule T del donatore (infuse insieme alle cellule staminali)



Reattive verso antigeni HLA minori del ricevente con effetto **antileucemico (GVL)**

DLI

DIPENDE

- Patologia di base
- Dose cellulare
- Timing di somministrazione
- Composizione cellulare

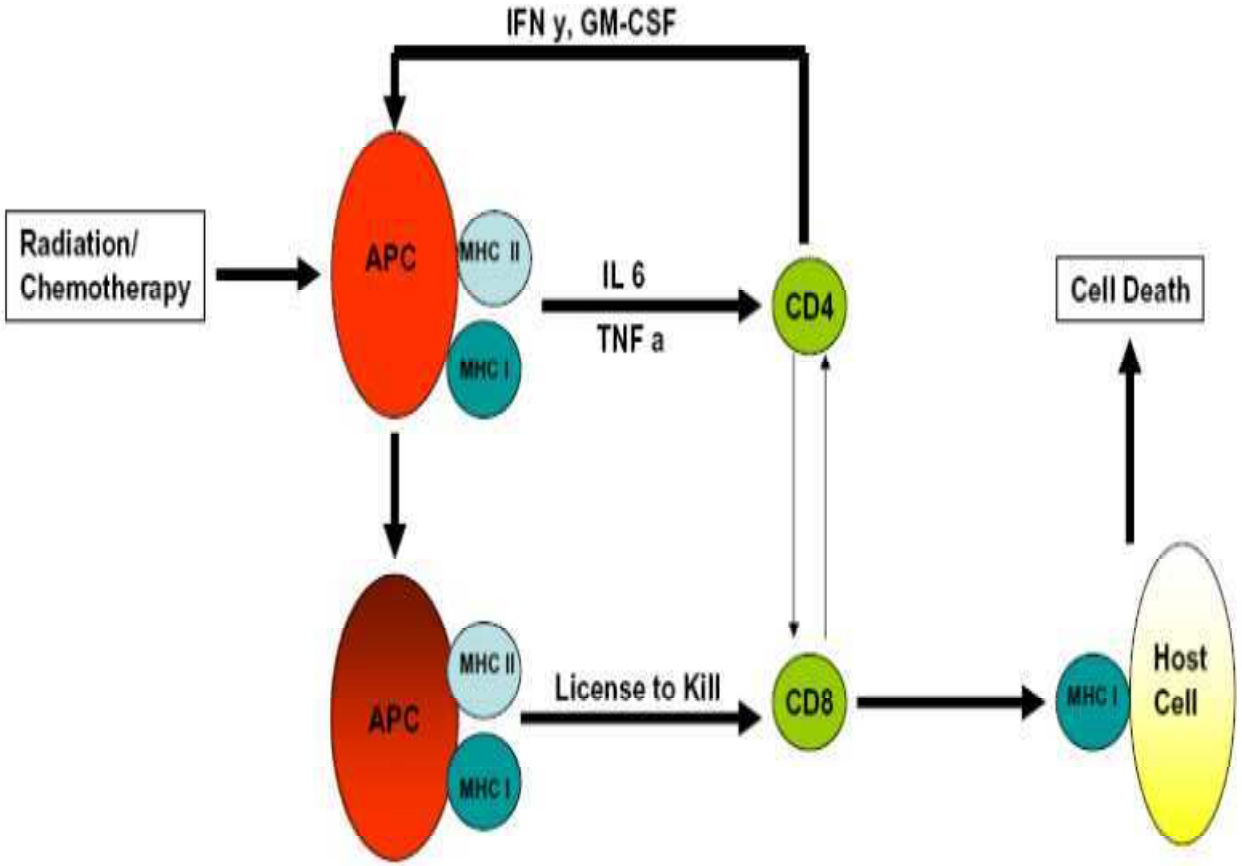


Fig. 1. Radiation/chemotherapy leads to damage to host cells, the antigen presenting cells of the host become activated and become highly proficient at presenting the antigens to the donor CD4/CD8 cells, which are able to costimulate and become effector cells which lead to tissue damage/anti-disease activity. The tissue damage leads to the release of cytokines which further accentuate the vicious cycle.

DLI

Effetto GVL (Graft vs.Leukemia)
mantenuto dalle cellule APC dell'ospite

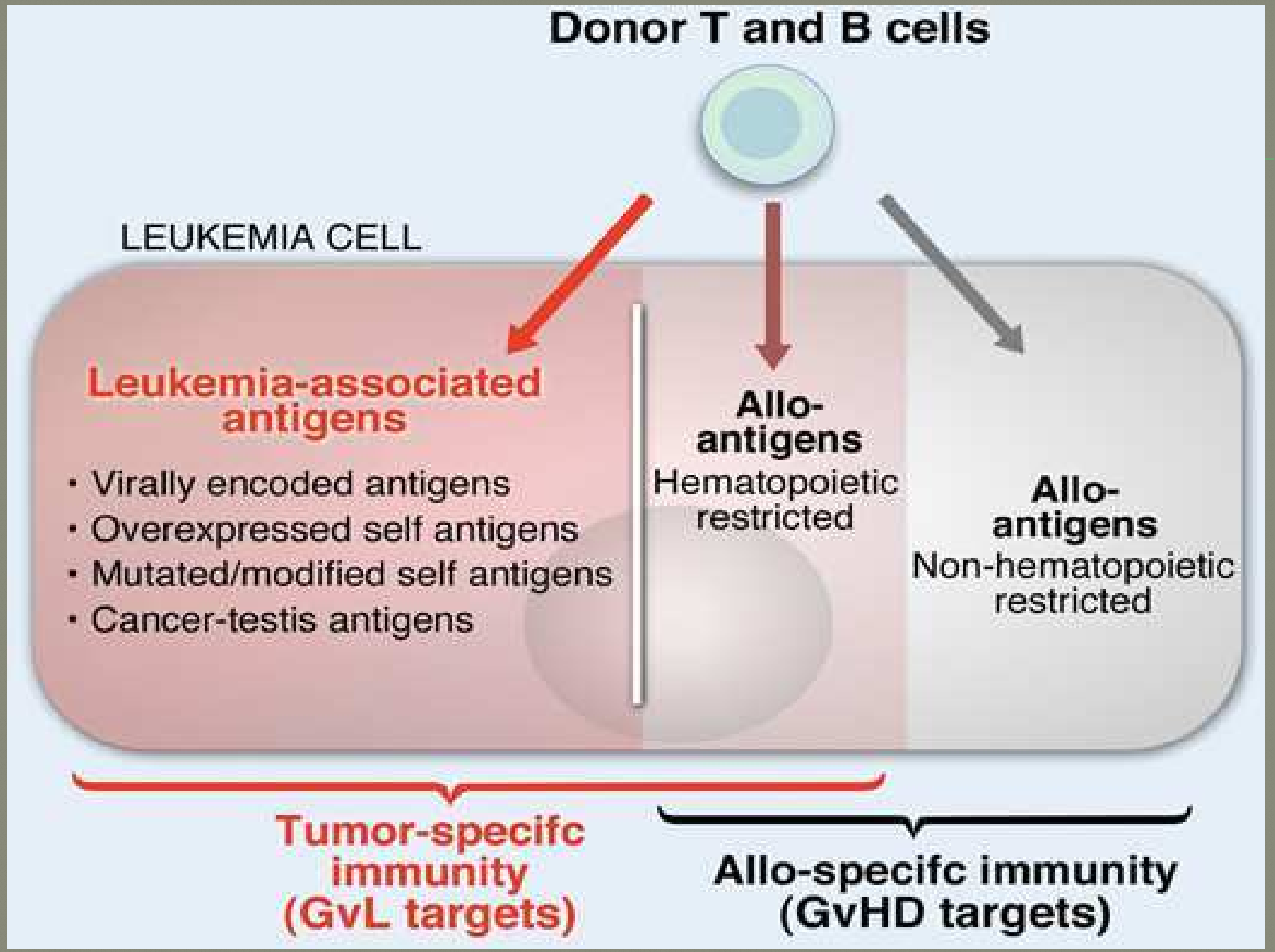
stimolano

T- cell del donatore presentando antigeni bersaglio espressi sulle staminali emopoietiche specifiche (mHA)

HA-1; HA-2; HB-1; BCL2-A1

DLI: effetto GvHD

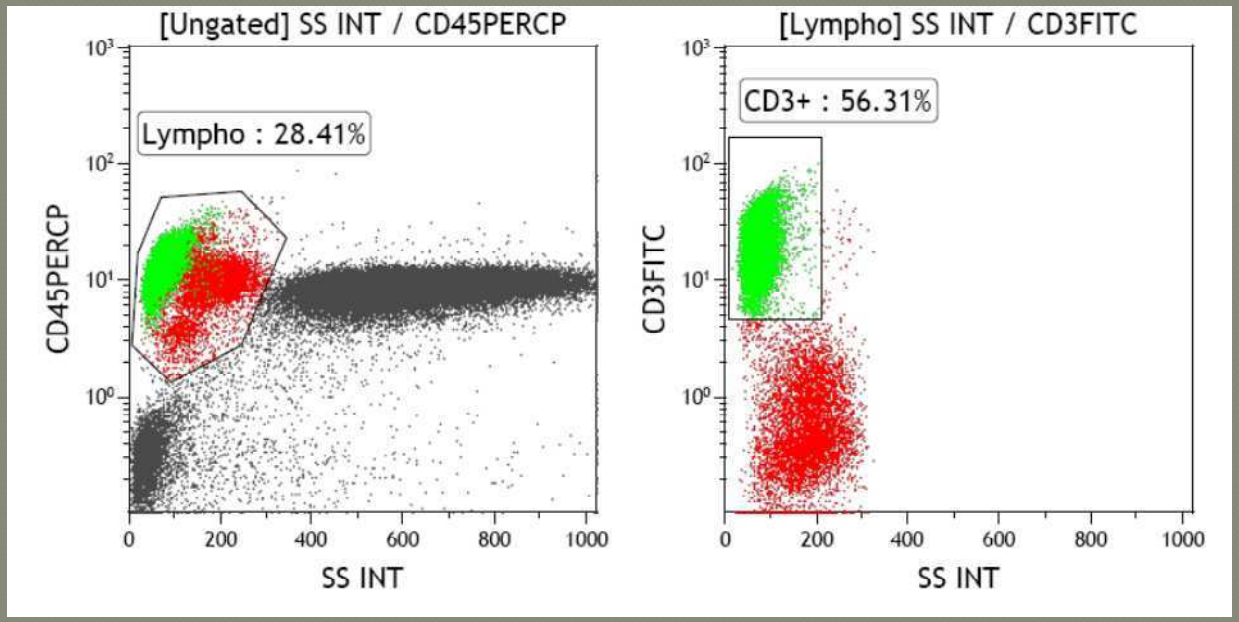
- Incidenza acuta GvHD: 8-48% (BDR-EDR)
- Incidenza cronica GvHD: 9-46%


Donor T and B cells
LEUKEMIA CELL
Leukemia-associated antigens
• Virally encoded antigens
• Overexpressed self antigens
• Mutated/modified self antigens
• Cancer-testis antigens
Allo-antigens
Hematopoietic restricted
Allo-antigens
Non-hematopoietic restricted
Tumor-specifc immunity (GvL targets)
Allo-specifc immunity (GvHD targets)

DLI

Quali linfociti?

CD3+



Le caratteristiche paziente /malattia SONO IMPORTANTI per la risposta

Table 1. Patient characteristics according to donor lymphocyte infusion regimen used

Features	Bulk (n = 28)	Escalating (n = 20)	Overall (n = 48)
Donor type			
SIB/VUD	18/10	10/10	28/20
Patient gender:			
Male/female	8/20	16/4	24/24
GVHD prophylaxis at SCT:			
TCD/T-replete	18/10	12/8	30/18
GVHD after SCT:			
Grade 2-4 acute	10	7	17
Extensive chronic	10	2	12
Relapse			
Cytogenetic alone	7	9	16
Hematologic-CP	17	6	23
Hematologic-AP	4	5	9
Interval SCT → relapse	16.6	12.2	12.5
Months (range)	3-103	4-51	3-103
Interval relapse → DLI	10.9	12.9	11.1
Months (range)	0-70	1-53	0-70

SIB, sibling donor; VUD, volunteer unrelated donor; SCT, stem cell transplant; GVHD, graft-versus-host disease; TCD, T-cell depleted SCT; hematologic-CP, chronic phase; hematologic-AP, hematologic relapse in advanced phase.

DLI: indicazioni

Leucemia mieloide cronica (rimane setting ideale)

- Risposta migliore nei pazienti con minore massa tumorale (tumor burden)
- **90%** dei paz. In remissione molecolare o citogenetica
- **60%** overall

Table 1. Response rates of chronic myeloid leukemia to donor lymphocyte infusions in major studies

Authors	Responders/total number of patients (%) at the time of donor lymphocyte infusion			
	Mrel/Cyrel	CP	AP	Overall
van Rhee et al. [18]	11/11 (100)	8/14 (57)	1/5 (20)	20/30 (66)
Collins et al. [19]	3/3 (100)	25/34 (73)	5/18 (27)	33/42 (78)
Drobyski et al. [20]	—	—	6/8 (75)	—
Porter et al. [21]	—	6/8 (75)	0/3 (0)	6/11 (54)
Kolb et al. [22]	14/17 (82)	39/53 (73)	1/14 (7)	54/84 (64)
Mackinnon et al. [23]	8/8 (100)	9/10 (90)	2/4 (50)	19/22 (86)
Bacigalupo et al. [24]*	6	12	N/S	10/18 (55)
Alyea et al. [25]	15/19 (79)		0/5 (0)	15/24 (62)
Verdonck et al. [26]	—	9/9 (100)	4/5 (80)	13/14 (93)
Sehn et al. [27]	N/S	N/S	N/S	19/23 (82)

AP indicates advanced phase (accelerated and blastic); CP, chronic phase; Cyrel, cytogenetic relapse; Mrel indicates molecular relapse; N/S, not specified.

*The number of patients at the time of treatment, not the number of responders per disease stage, was reported in this study.

DLI: indicazioni

Leucemia mieloide acuta e MDS

significativamente
meno efficace

LMA: 2 years OS: 21+- 3%

DLI: indicazioni

Mieloma multiplo

Overall response < 50%

Risposta prevista con 100x10e6 CD3+/kg

DLI: indicazioni

- Leucemia acuta linfoide
- LH
- LnH

Dose escalating: 20x10e6 CD3+/kg

Risultati < 50% overall response

Table 5
Multiple myeloma studies.

Authors	Number of patients	Graft source RD/UD	Median time to relapse from SCT	Other treatments	Mean number of DLI	DLI dose Median T cells/kg (range)	Disease state after DLI	Survival
Collins et al.[47]	5	5/0	NA	1 PR, 4 refractory disease	2	$(1.0–8.2) \times 10^8$	50% CR	40% at 2 years
Lokhorst et al.[23]	13	9/0	5–87 months	6 PR, 7 refractory disease	NA	$(0.01–3.3) \times 10^8$	62% response, 31% CR	5–38 months
Lokhorst et al.[84]	27	NA	30 months	13 pts. got chemo	NA	$(0.01–5) \times 10^8$	52% response, 30% CR	40% at 5 years (70% for responders)
Salama et al.[24]	25	24/1	57.5 months	4 pts. got chemo	9 pts. got 2	$(0.02–5.55) \times 10^8$	36% response, 28% CR	48% at 1 year
van de Donk et al.[85]	63	46/17	Included persistent and relapsed dis	None	7 got more than 1	$(0.01–3) \times 10^8$	38% response	50% at 2 years (62% for responders)

Abbreviations: RD, related donor; UD, unrelated donor; CML, chronic myeloid leukemia; NA, information not available; CR, complete remission; DLI, donor lymphocyte infusion; SCT, stem cell transplant.

Table 1 Studies of therapeutic DLI that included multiple diseases

Study/follow-up after DLI	*n*	*Diseases*	*Donor source (N)*	*Cell dose ($\times 10^8$) cells are CD3+ T cells unless noted (N)*	*aGVHD grade II–IV*	*cGVHD*	*Response*	*OS*	*PFS/LFS*	*TRM*
Collins[7] 194 days	140	CML = 56; AML = 46; ALL = 15; MDS = 6; NHL = 6; MM = 5; Other = 6	MRD (121) MmRD (7) URD (12)	220 ± 120/kg (26) 70 ± 297 MNC/kg (78) 480 ± 344 TNC/kg (29) Unknown (7)	58/125	51/84	CR: 45/140 (CML = 33; AML = 6; ALL = 2; MDS = 2; MM = 2)	NR	NR	At 1 year 14% (8–20)
Raiola[5] 15 months	100	CML = 51; AML = 19; ALL = 10; LPD = 10; MPD = 3; MDS = 7	MRD (87) MmRD (7) URD (6)	BDR: >50/kg (n = 14) ESD: median number of DLI = 4; median total dose = 76/kg (n = 86)	21/100	6/100	CR: 47/100 (CML = 35/51; other = 12/49)	At 10 years 36%	At 5 years (for those obtaining CR) 85%	9/100
Huff[8] CML: 857 days Other: 383 days	83	CML = 22; MM = 20; AML = 13; MDS = 12; ALL = 3; HL = 8; NHL = 5	MRD	CML: 10/kg MM, MDS = 50/kg AML/ALL, HL, NHL = 100/kg dose escalation permitted	29/83	27/83	ORR: 37/83 (CML = 14; MM = 8; AML = 6; MDS = 2; ALL = 1; HL = 3; NHL = 3	CML CP/cyto rel at 2 years: 16/17 Others at 1 year: 9/66	NR	22/83
Chiorean[3] 59 months	42	CML = 24; AML = 10; MDS = 6; ALL = 1; JMML = 1	MRD (34) MmRD (1) URD (7)	CML: 100/kg Other diseases: total of x3 lympho-apheresis	20/42	14/42	CR: 25/42 (CML = 18; AML = 6; MDS = 1)	CML at 5 years 75% (58–92) Other at 2 years 17% (0–34)	NR	NR
Michallet[10] 58 months	30	CML = 14; AML = 9; ALL = 4; NHL = 1; MDS = 1; MM = 1	MRD (27) URD (3)	BDR: 10–264/kg (n = 10) ESD: 1–300/kg (n = 20)	4/30	3/30	ORR: 18/30 (CML = 14; AML = 2; ALL = 1; NHL = 1)	At 3 years CML: 80% Other: 48%	NR	NR
Schattenberg[16] 47 months	26	CML = 15; AML = 6; ALL = 4; MDS = 1	MRD	70/kg multiple DLI (9) median total dose: 110/kg	8/26	10/19	CR: 12/26 (CML = 11; AML = 1)	NR	NR	NR
Peggs[20] NR	46	MM = 19; HL = 13; NHL = 10; CLL/PLL = 3; CML = 1	MRD (32) URD (14)	ESD: 1/kg (33); 3/kg (23); 10/kg (26); 30/kg (16); 100/kg (10) Given every 3 months	12/45	10/41	ORR: 21/34 CR: 9/34 (MM = 1; HL = 5; NHL = 1; CLL = 1; CML = 1)	NR	NR	5/46
Verdonck[4] NR	28	CML CP = 9; CML AP/BC = 5; MM = 5; AL = 9	MRD (28) URD (2)	1–330/kg multiple DLI (8)	18/28	16/21	ORR: 19/28 (CML = 13; AL = 1; MM = 5)	NR	NR	6/28

Abbreviations: aGVHD = acute GVHD; AL = acute leukemia; AP = accelerated phase; BC = blast crisis; BDR = bulk dosing regimen; cGVHD = chronic GVHD; CP = chronic phase; ESD = escalated dosing regimen; HES = hypereosinophilic syndrome; HL = Hodgkin's lymphoma; JMML = juvenile myelomonocytic leukemia; LPD = lymphoproliferative disorder; MDS = myelodysplasia; MF = myelofibrosis; MM = multiple myeloma; MmRD = mismatched related donor; MNC = mononuclear cells; MPD = myeloproliferative disorder; MRD = matched related donor; NHL = non-Hodgkin's lymphoma; NR = not reported; OS = overall survival; PLL = prolymphocytic leukemia; RIC = reduced intensity conditioning; TNC = total nucleated cell dose; TRM = treatment-related mortality; URD = unrelated donor.

DLI

Mortalità

5-20%

> 1/3 pazienti svilupperà GvHD (acuta o cronica)

DLI: cell dose

0,01-8.8 x 10e8/kg

Dose inferiore a 1x10e8 si può definire sub ottimale

Dose superiore a 4,5x10e8 non ulteriore vantaggio

DLI: cell dose
strategia di somministrazione

- Bulky dose (1.5 x10e8 median dose)

- Escalating dose : a) riduzione rischio GvHD
  b) dose totale linfociti maggiore

1-5 x10e6; 1x10e7; 5x10e7; 1x10e8.....

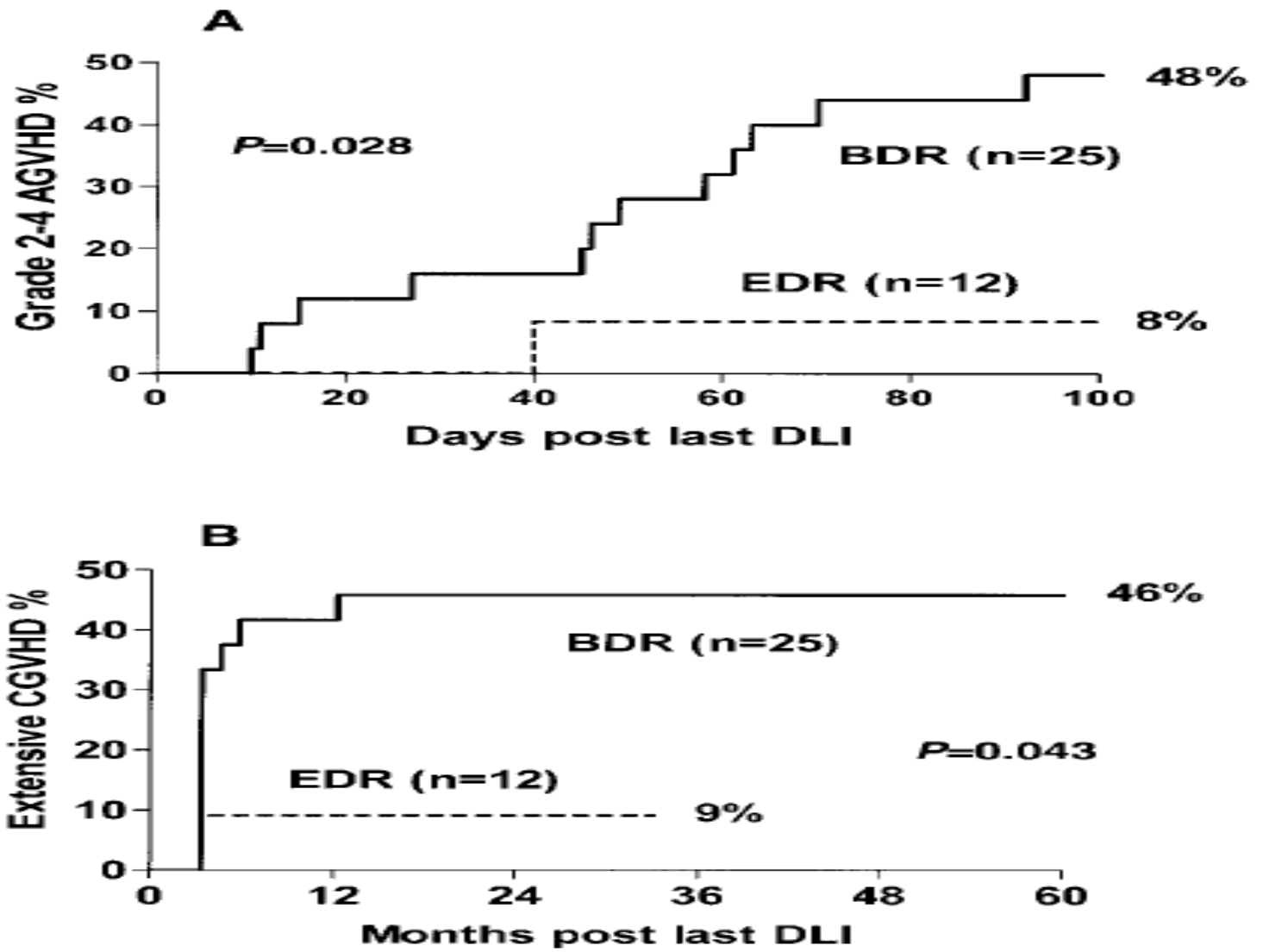


Figure 2. Probability of acute and chronic graft-versus-host disease. Probabilities of developing grade 2-4 acute GVHD (A) and extensive chronic GVHD (B) in 37 patients treated by escalating-dose or bulk-dose infusion regimens of donor lymphocytes in the 0.4 to 3.3 $\times$ 10^6/kg range.

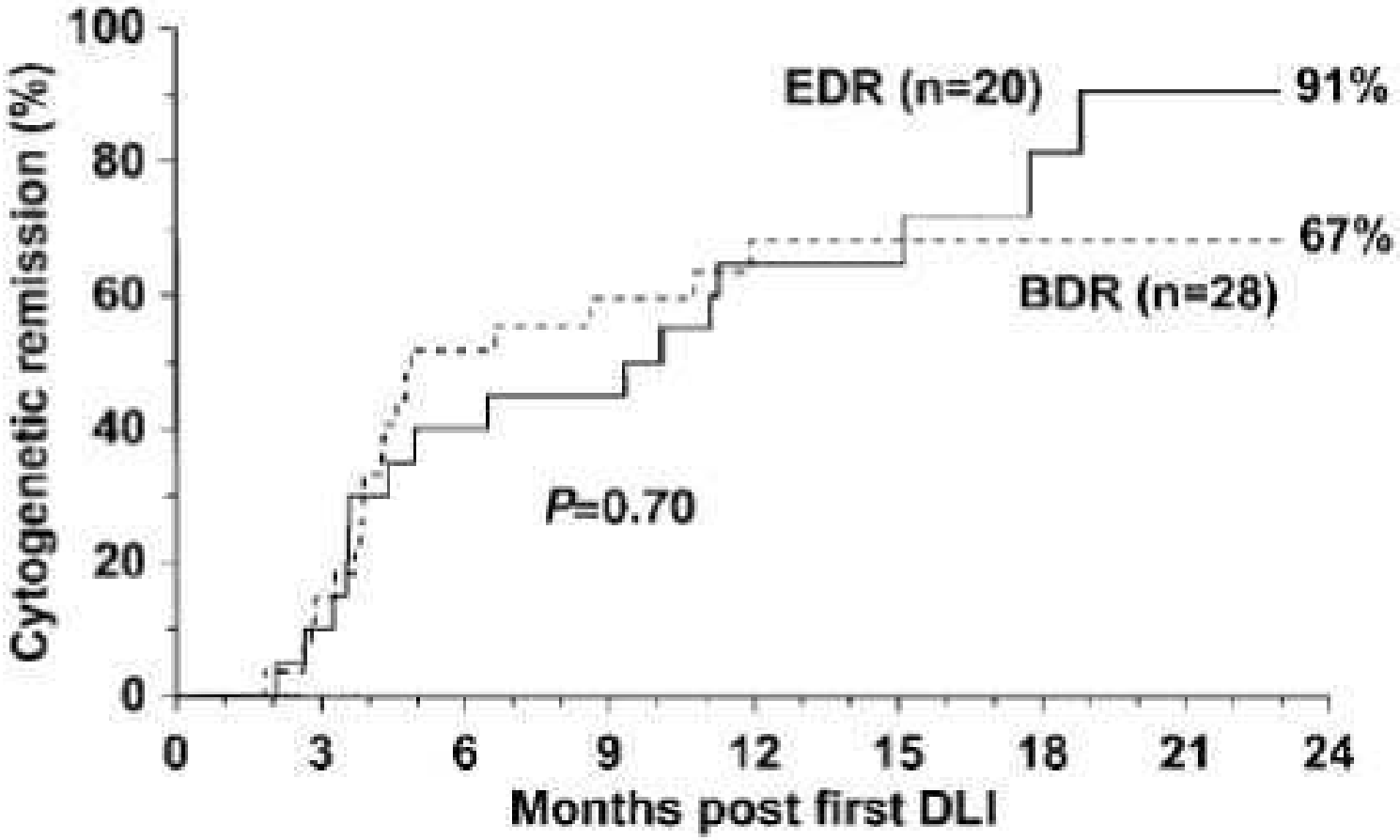


Figure 1. Probability of cytogenetic remission. Probability of achieving cytogenetic remission for 48 patients who received escalating-dose or bulk-dose infusion regimens dated from the first (or only) infusion of donor lymphocytes.

tDLI e pDLI

- tDLI : terapeutica (per curare la ricaduta)

- pDLI: pre-emptive (pianificata per minimizzare la ricaduta neoplastica)

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Table 2 Studies of pre-emptive DLI which included multiple diseases

Study/follow-up	n	Diseases	Donor source/ conditioning	Cell dose ($\times 10^6$) cells are CD3+ T cells unless noted	aGVHD grade II–IV	cGVHD	Relapse	OS	PFS/LFS	TRM
Barrett[20] 20.5 months	38	Std risk: CML CP = 13; CML AP = 3; AML = 2 High risk: CML BC = 3; tMDS = 5; AML = 4; MM = 8	Related marrow TCD MA	Group 1 (n = 26): day +30 = 2/kg; day +45 = 5/kg Group 2 (n = 12): day +30 = 10/kg	Group 1: 31.5% Group 2: 100%	10/24 (46%)	Std risk: 29 ± 13% High risk: 69 ± 23%	45.5 ± 8.5%	Std risk: 72 ± 9% High risk: 12 ± 10%	16/38 (42%)
Schaap[21] 3 years	82 (35 DLI)	AML = 28; ALL = 10; CML = 27; MDS = 7; MM = 10	Related marrow TCD MA	High dose (n = 6): 70/kg Low dose (n = 25): 10/kg	High dose: 5/6 patients Low dose: 8/25 patients	NR	DLI: 18% (5–31) No DLI: 44% (28–60)	DLI: 79% (63–95) No DLI: 63% (47–79)	DLI: 77% (63–91) No DLI: 51% (36–66)	2/35 (6%)
Montero[22] 47 months	138 (112 DLI)	Std risk: AML CR1 = 17; ALL CR1 = 5; CML CP1 = 42; MDS RA = 10; other = 3 High risk: AML > CR1 = 21; ALL > CR1 = 16; CML AP/BC = 12; MDS > RA = 8; other = 4	Related marrow TCD MA	10/kg Given between day +45 and day +100 Single DLI (n = 70) Two DLI (n = 42)	53/138 (38%)	71/116 (61%)	20 ± 5%	58 ± 5 %	46 ± 5%	20 ± 4%
Ferra[23] 585 days	22 (12 DLI)	CML = 10; AML = 6; ALL = 3, MDS = 1, CLL = 1; HD = 1	Related PBSC CD34 selected MA	Day +28 = 0.2/kg (n = 12) day +60 = 0.2/kg (n = 6) day +90 = 2/kg (n = 3)	28% (7–49)	38% (10–63)	54% (29–79)	65% (43–86)	33% (11–55)	3/22 (14%)
Nakamura[24] 510 days	51 (44 DLI)	CML CP = 13; CML AP = 4; AML = 14; MDS = 11; ALL = 4; MM = 2; CLL = 2; NHL = 1	Related PBSC TCD MA	Day +45 = 10/kg (n = 44) day +100 = 50/kg (n = 31)	33 ± 7%	21/46 (46%)	39 ± 10%	51 ± 10%	47 ± 9%	6 ± 3% (day 100)
Lee[20] 3 years	52 (38 DLI)	DLCL = 15; FL = 22; MCL = 4; Mediastinal NHL = 2; lymphoblastic = 4; Burkitt = 2; HL = 3	Related (33) URD (19) TCD MA	Varied based on patients GVHD risk category[a] 1 infusion = 38 2 infusions = 17 3 infusions = 5	1 DLI: 14/38 2 DLI: 9/17 3 DLI: 2/5	20/32 (63%)	23 ± 8%	34 ± 7%	34 ± 7%	27/52 (52%)

Abbreviations: aGVHD = GVHD; AP = accelerated phase; cGVHD = chronic GVHD; CP = chronic phase; DLCL = diffuse large-cell lymphoma; FL = follicular lymphoma; HD = Hodgkin's disease; MA = myeloablative conditioning; MCL = mantle cell lymphoma; MM = multiple myeloma; NHL = non-Hodgkin's lymphoma; NR = not reported; OS = overall survival; RA = refractory anemia; Std = standard; TCD = T-cell depleted; tMDS = therapy-related myelodysplastic syndrome; TRM = treatment-related mortality.

[a]GVHD Risk category: low risk = matched-related donor, ⩽35 years; intermediate risk = matched-related donor, 36-50 years; matched URD ⩽50 years; High risk = all other patients.

DLI: selezione del donatore

- Rivalutazione del soggetto donatore (legge italiana; linee guida GITMO)

Studio funzionalità cardiaca (ECG, EcoCardiogramma), renale, epatica, emocromo completo di formula leucocitaria, virologia, lue, test di gravidanza.

esami eseguiti entro 30 gg dalla data di raccolta
studio preventivo accessi venosi periferici

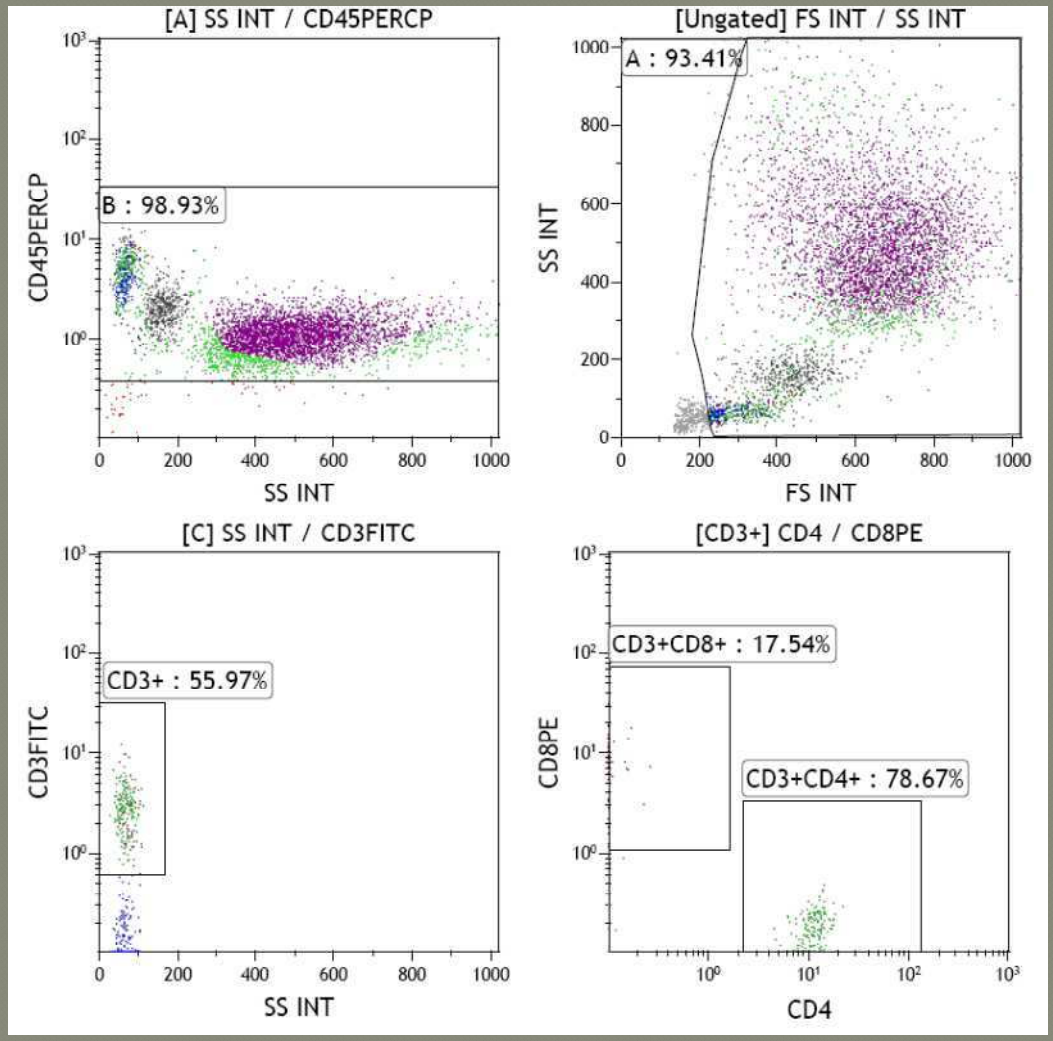
DLI: raccolta aferetica

- Separatore cellulare ultima generazione
- Pre-calcolo (approssimativo) della dose di linfociti da raccogliere
- Programma MNC-linfociti
- Durata procedura (max 100min.)
- HCT sacca desiderabile 2-4%
- Raccolta granulocti minima

DLI: il prodotto

- Etichettatura univoca
- Emocromo sacca e citofluorimetria in tempo reale
- Ispezione sacca per corretta anticoagulazione e integrità
- Tracciabiltà percorso da aferesi a laboratorio
- Richiesta scritta caratteristiche minime prodotto

DLI: citofluorimetria

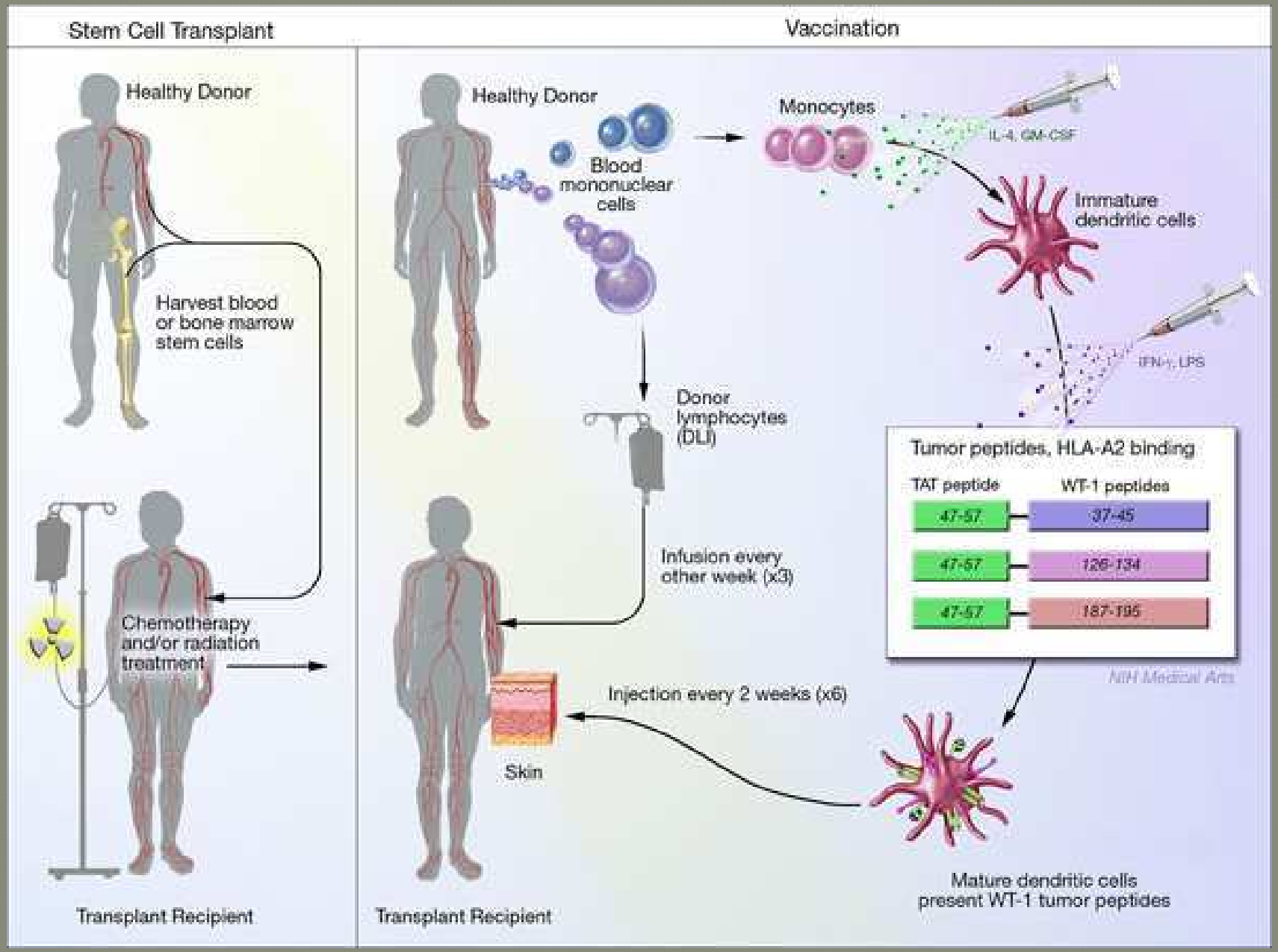


DLI: infusione

- Ricontrollo rigoroso della dose cellulare da infondere
- Sorveglianza del paziente durante l'inoculo
- Follow up clinico e bio-umorale

DLI: nuove strategie

- Allogenico con selettiva T-deplezione (CD8+)
- Educazione anti virale (autologo): anti EBV; CMV; polioma virus
- Educazione anti fungina (autologo): aspergillus
- Anti neoplastica (autologo-allogenico): programmi di vaccinazione antitumorale


Stem Cell Transplant
Vaccination
Healthy Donor
Harvest blood or bone marrow stem cells
Chemotherapy and/or radiation treatment
Transplant Recipient
Healthy Donor
Blood mononuclear cells
Donor lymphocytes (DLI)
Infusion every other week (x3)
Skin
Injection every 2 weeks (x6)
Transplant Recipient
Monocytes
IL-4, GM-CSF
Immature dendritic cells
IFN-γ, LPS
Tumor peptides, HLA-A2 binding
TAT peptide
WT-1 peptides
47-57
37-45
47-57
126-134
47-57
187-195
NIH Medical Arts
Mature dendritic cells present WT-1 tumor peptides

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Cell Therapy of Stage IV Nasopharyngeal Carcinoma With Autologous Epstein-Barr Virus–Targeted Cytotoxic T Lymphocytes

Patrizia Comoli, Paolo Pedrazzoli, Rita Maccario, Sabrina Basso, Ornella Carminati, Massimo Labirio, Roberta Schiavo, Simona Secondino, Chiara Frasson, Cesare Perotti, Mauro Moroni, Franco Locatelli, and Salvatore Siena

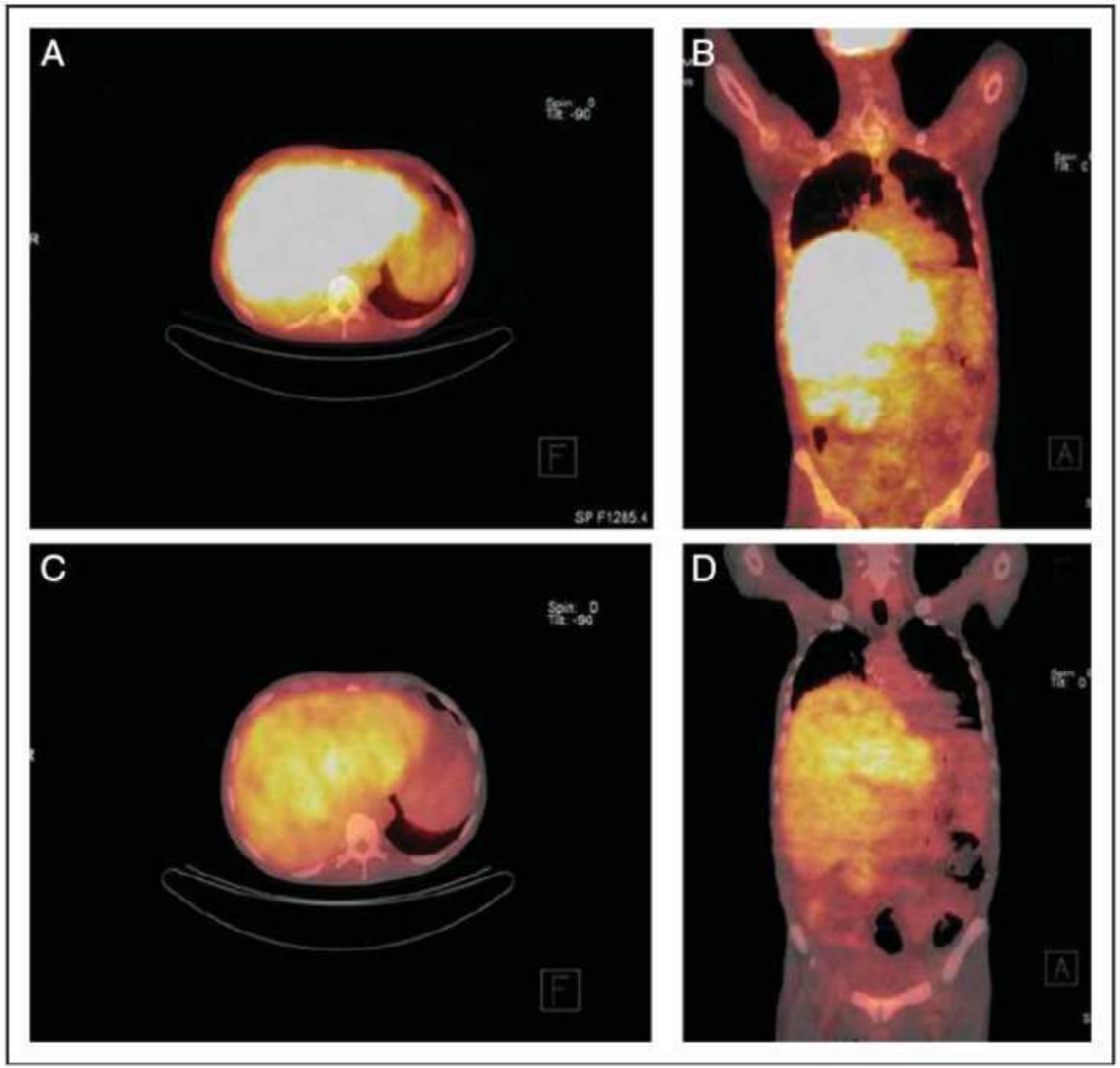

Fig 2. Objective response to Epstein-Barr virus (EBV) –targeted cytotoxic T-lymphocyte (CTL) therapy in patient 7. (A and B) Computed tomography–aided positron emission tomography imaging before T-cell therapy shows massive liver involvement. (C and D) After 3 months of EBV-targeted CTL therapy, reduction of size and isotope uptake of NPC lesions was detected.

GRAZIE!