

PROGRAMMA PER LA RICERCA ONCOLOGICA

“Rete solidale e collaborazioni internazionali” (DM del 21 luglio 2006, Art. 3, ISS per ACC)

DICHIARAZIONE DI INTENTI

Proposta di progetto per il programma 3: trasferimento delle conoscenze allo sviluppo di interventi volti a prevenire, diagnosticare e trattare il cancro (trials nazionali in terapie innovative e in prevenzione, e in terapie non d’interesse industriale in collaborazione con aifa).

Titolo della proposta

Applicazione della chemioterapia alla rimodulazione della risposta immune antitumorale: studio dei meccanismi e “proof of concept” nell’uomo

Area tematica della proposta

Area tematica 2. Bioterapie dei tumori

Unità Operativa 1

(Unità di Coordinamento della proposta)

Coordinatore Scientifico Enrico Proietti, Istituto Superiore di Sanità

Gruppi di Ricerca afferenti al DI proponente

ISS, Dip. Malattie Infettive, Patogenesi Molecolare, **Angela Battistini**

ISS, Dip. Ambiente e Prev. Primaria, Cancerogenesi Sper., **Margherita Bignami**

ISS, Dip. Farmaco, Farmacogenetica e Farmacoresistenza, **Stefano Fais/ Franca Podo**

ISS, Dip. Biol. Cellul. e Neuroscienze, Immunoterapia, **Lucia Gabriele/ Eleonora Aricò**

Unità Operativa 2

Coordinatore Scientifico Paola Nisticò, IRE

Gruppi di Ricerca afferenti al DI proponente

IRE- Lab. Immunologia Patologia Molecolare, **Piergiorgio Natali**

IRE- Oncologia Medica A, **Virginia Ferraresi**

Gruppi di Ricerca afferenti ad altre Istituzioni di Ricerca

Istituto S. Gallicano, **Caterina Catricalà**

Dip. Neuroscienze, Fac. Di Med. e Chir., Univ. di Roma Tor Vergata, **Ornella Franzese**

Unità Operativa 3

Coordinatore Scientifico Marco Bregni, Istituto Scientifico Fondazione San Raffaele (HSR)

(attualmente si è trasferito all'ospedale San Giuseppe – Milano)

Gruppi di Ricerca afferenti ad altre Istituzioni di Ricerca

Istituto Nazionale Tumori (Milano) **Licia Rivoltini**

Istituto Europeo di Oncologia **Maria Rescigno**

PREMESSA

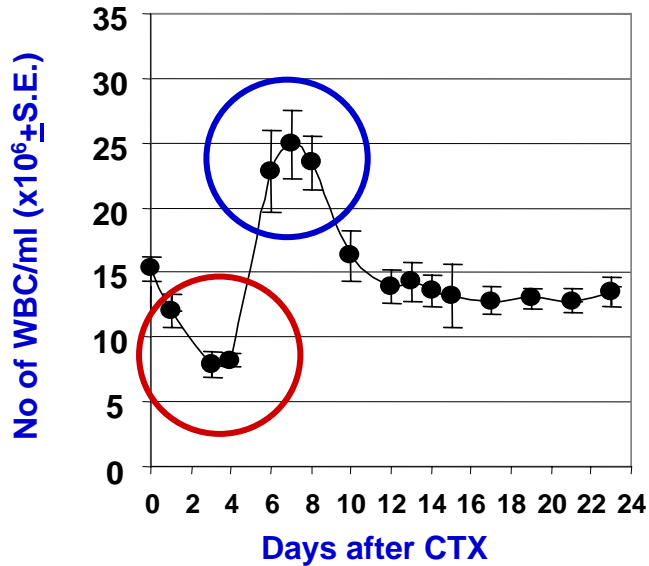
L'importanza del microambiente sulla crescita tumorale è ormai ampiamente riconosciuta, tuttavia la maggior parte degli interventi terapeutici nel cancro è ancora disegnata per agire esclusivamente in modo diretto contro le cellule tumorali.

Solo di recente, la ricerca si è orientata verso la comprensione delle interazioni tumore/ambiente (stroma, endotelio vascolare, “immune escape”).

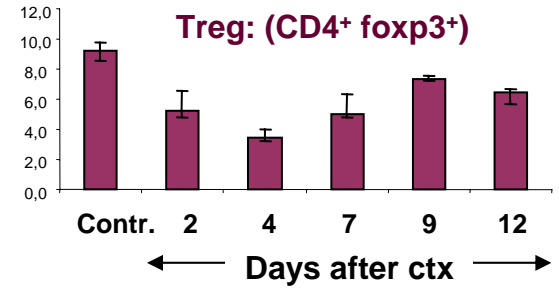
In questa ottica è possibile riconsiderare l'uso di alcuni chemioterapici per i loro effetti sull'organismo ed in particolare sul sistema emopoietico e linfopoietico, a prescindere dalla loro azione diretta contro il tumore.

Diversi studi hanno dimostrato che la chemioterapia antineoplastica può essere vantaggiosamente combinata con strategie di immunoterapia.

mechanisms involved (murine models)



Brush



Homeostatic proliferation

CTX

CTX treatment selectively reduces T reg cell number

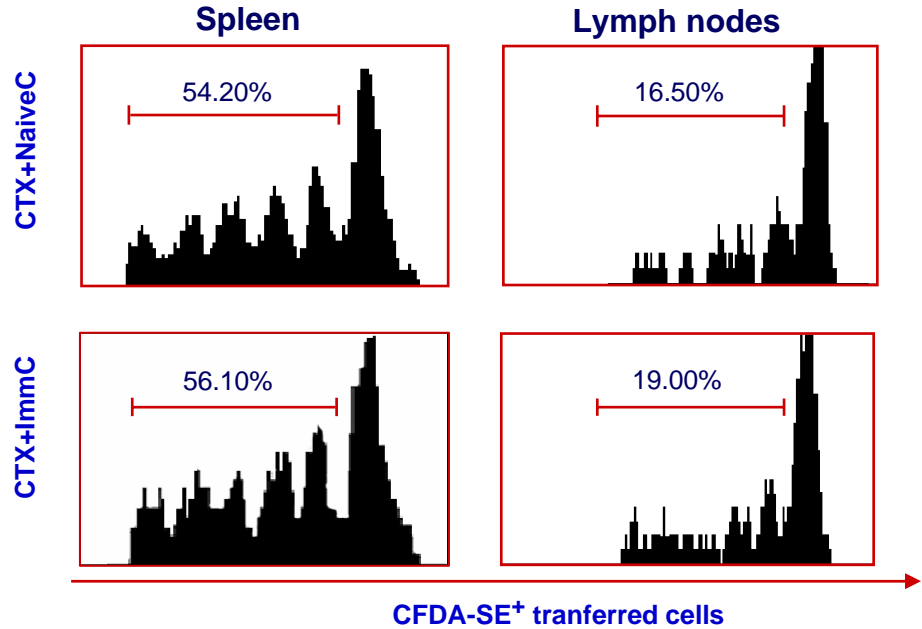
Pace et al. man. In prep.

CTX treatment discontinuation induces a rebound overshoot

Greco et al. J. Clin. Inv. 1998

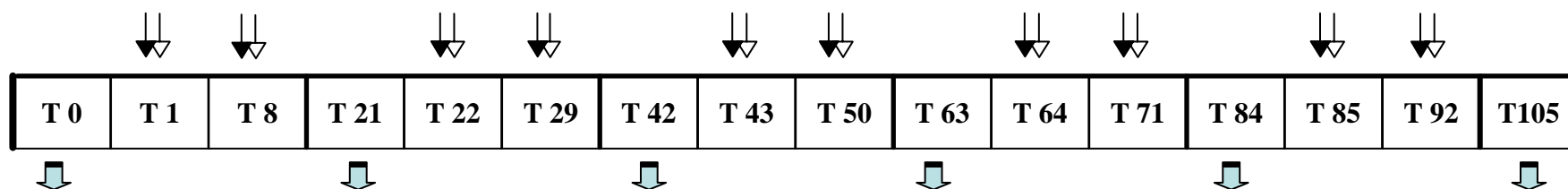
Transferred cells undergo homeostatic proliferation

Bracci, Moschella et al. Clin Cancer Res. 2007

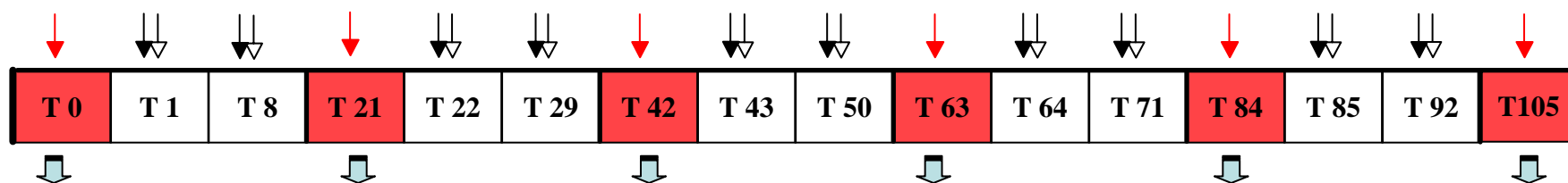


Clinical study on Dacarbazine-vaccine combination in resected melanoma patients


Arm A Peptide vaccine alone (5 pts)



Arm B Vaccine + Dacarbazine (5pts)



 = DTIC: Dacarbazine (Deticene[®], 800 mg/m²)

 = Interferon alpha: (Alfaferone[®] (3x10⁶ IU)

 = Peptides (250 µg x 2) in Montanide ISA 51

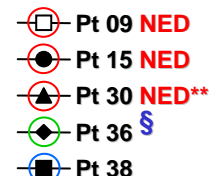
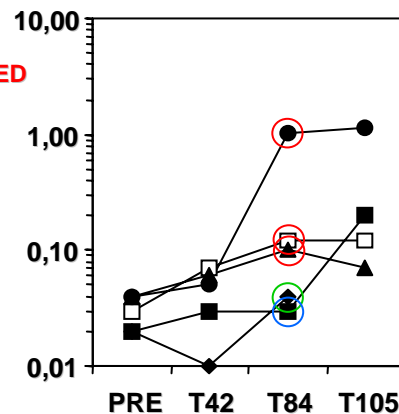
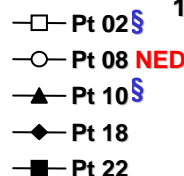
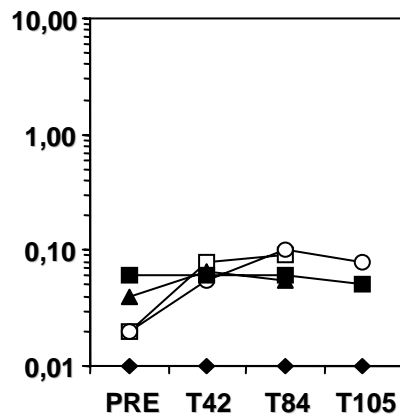
 Monitoring of Ag-specific T cells

Ex vivo CD8⁺ T cell response to Melan-A and gp100 peptides during vaccination

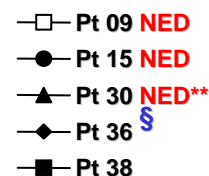
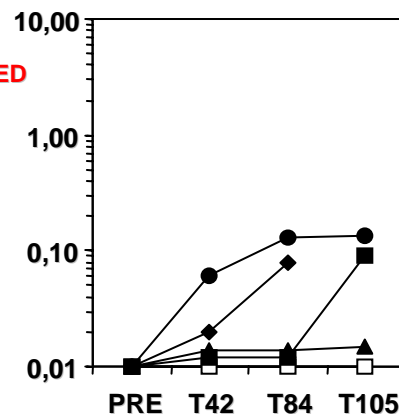
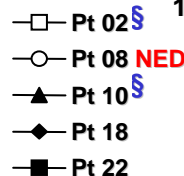
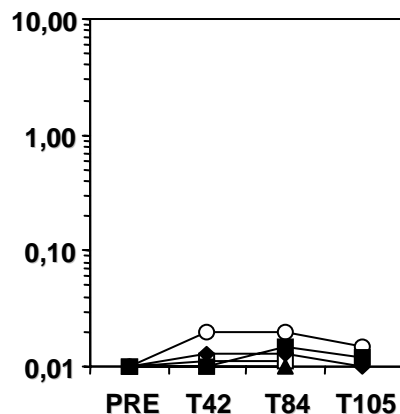
ARM 1 (vaccine alone)

ARM 2 (vaccine + DTIC)

% A2/Melan-A-tetramer⁺specific T cells



% A2/gp100-tetramer⁺specific T cells



Vaccination Course

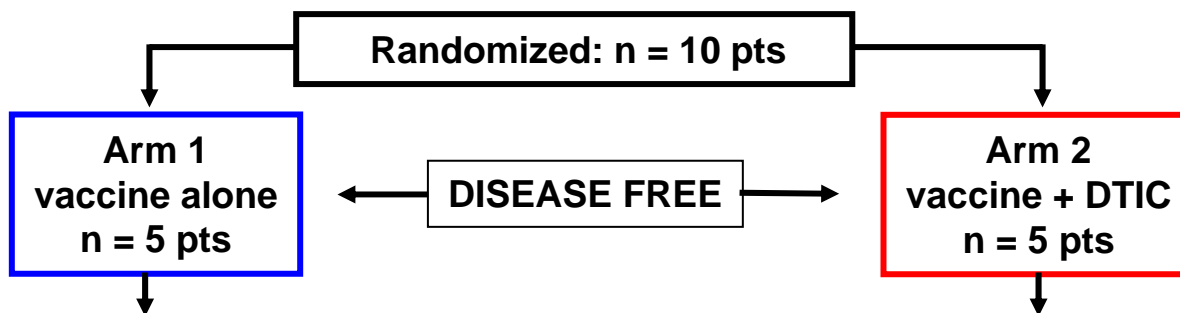
Vaccination Course

§ Discontinued due to disease progression

NED: No evidence of disease (2-2.5 years after treatment)

**disease-free following second line chemotherapy

Start: Nov. 2004
End: Aug. 2005



Pt#	Arm	Stage	Previous therapy	Disease sites	RFS (mo) [§]	Site of relapse	OS (mo)
02	1	IV	S, BT	Lu	3	Brain	45+
08	1	II	S	-	45+	-	45+
10	1	IV	S, BT	Lu	2	Lu, LN	7
18	1	IV	S, CT, RT	LN	6	LN	45+
22	1	III	S	-	8	Brain	24

Pt#	Arm	Stage	Previous therapy	Disease sites	RFS (mo) [§]	Site of relapse	OS (mo)
09	2	III	S	-	47+	-	47+
15	2	IV	S	ST	45+	-	45+
30	2	III	S	-	41	Lu [¶]	41+
36	2	IV	S, BT	Lu	2	Bone	9
38	2	III	S	-	4	Liver	9

* Arm 1, vaccine alone; Arm 2, dacarbazine plus vaccine. No grade (G) 3/4 WHO criteria hematological and no hematological toxicities were observed

† S, Surgery on metastatic lesions; BT, biotherapy; CT, chemotherapy; RT, radiotherapy

‡ Lu, lung; LN, lymph nodes; ST, soft tissue

§ RFS, relapse-free survival

¶ This patient is presently disease-free following second line chemotherapy

** OS, overall survival

Applicazione della chemioterapia alla rimodulazione della risposta immune antitumorale: studio dei meccanismi e “proof of concept” nell’uomo

Obiettivo principale

Il progetto prevede il disegno e la realizzazione di due trial clinici di chemio- immunoterapia.

Il primo studio (UO 1, UO 2) prevede una vaccinazione peptidica associata a trattamento con chemioterapia.

Lo studio sarà eseguito in pazienti con **melanoma in stadio III e IV** in cui il tumore è stato rimosso chirurgicamente.

Durante la fase di vaccinazione sarà possibile lo studio e la standardizzazione dei correlati immunologici della risposta antitumorale e la definizione dei profili genici indotti dai trattamenti chemioterapici.

Questo studio fornirà le cognizioni di base organizzative per procedere ad un secondo studio in cui il vaccino sarà costituito da DC autologhe caricate con corpi apoptotici del tumore

Il secondo studio (UO3) prevede il trattamento di pazienti affetti da **ca ovarico e da ca coloretale** con linfociti autologhi educati ex vivo contro il tumore. Tali linfociti saranno isolati dal sangue dei pazienti ed educati in vitro con opportune metodiche colturali. Raggiunto il numero sufficiente i **linfociti saranno reinfusi** i.v. nello stesso paziente **dopo trattamento linfopenizzante** con Cy e fludarabina.

Anche in questo studio saranno valutati i correlati immunologici della risposta antitumorale, il ruolo di meccanismi immunosoppressori e l’impatto dei polimorfismi di geni immuno-relati sulla modulazione della risposta al vaccino.

Studio 1: definizione del protocollo clinico

STUDY PROTOCOL

Title	Peptide-based vaccine in combination with chemotherapy in melanoma patients: a phase II randomized clinical study.
Study code	ISS-DPII
Study phase	II
Sponsor	Istituto Superiore di Sanità
Scientific Coordinator	Enrico Proietti, MD Istituto Superiore di Sanità Rome, Italy Paola Nisticò Regina Elena Cancer Institute Rome, Italy
Principal Investigator	Virginia Ferraresi, MD Regina Elena Cancer Institute Rome, Italy
Co-Investigators	Caterina Catricalà, MD S. Gallicano Dermatological Institute Rome, Italy Filippo Belardelli, PhD Istituto Superiore di Sanità Rome, Italy Patrizio Pezzotti, PhD Scientific Direction Public Health Agency of Lazio Via S. Costanza, 53 00198 – Rome, Italy Tel: 0683060342; e-mail: pezzotti@asplazio.it

4 STUDY DESIGN

This is a phase II, open-label, randomized study which evaluate in stage III and IV (with resected tumor) melanoma patients, a vaccination treatment versus a combined chemo-vaccine treatment. At least 50 subjects (25/arm) will be enrolled to receive the two treatments. Subjects will be asked to sign an informed consent form before entering the study.

Patients will be assigned to two treatment arms. Patients in arm I will receive i.d. injections of Melan-A/MART-1 and NY-ESO 1 peptides (250 µg each), formulated in Montanide ISA-51, plus s.c. injection of 6 MU interferon-α (IFN-α) as an immune adjuvant. The treatment includes 6 administration courses, each course repeated every 21 days and comprising two vaccine/IFN injections on days 1 and 8. Patients in arm II will receive the same vaccination schedule combined with DTIC (800 mg/mq i.v.) administered one day before each vaccination course (i.e every 21 days).

Before treatment and after the 4th and 6th treatment course as well as at 6th month from the beginning of the treatment blood samples will be taken for determining safety as well as efficacy parameters. Additional small blood samples will be taken after each remaining treatment course for determining only specific safety parameters and T-cell response (as illustrated in appendix 1).

During treatment, patient clinical status will be monitored at each visit and any disease recurrence will be checked by appropriate diagnostic investigations at specific time points. After the end of the treatment, a two years follow-up period is foreseen. Patients showing any relapse during the treatment period will be withdrawn from the study and will not be considered assessable. We estimate 24 months to reach 50 subjects who will be assessable for primary as well as secondary endpoints. The expected duration of the study is 4 years.

5 STUDY POPULATION

Stage IIIb (with macrometastases, any Nc) and IIIc or IV M1a or M1b, according to the AJCC classification, disease-free melanoma patients will be enrolled in the study. Patients without evidence of disease, as documented by CT scan performed within 30 days before randomization, will be enrolled. Selection of the study population will be carried out at the following centers: Regina Elena Cancer Institute and S. Galliciano Dermatological Institute.

5.1 SUBJECT INCLUSION CRITERIA

- Histologically confirmed AJCC stage IIIb or IIIc or IV (M1a or M1b) disease-free HLA-A*0201melanoma;
- Adult subjects of ≥18 years of age;
- ECOG score 0-1;
- Life expectancy of at least 6 months;
- Hematopoietic, liver and renal normal functions defined as follows: WBC ≥ 3 x 10³/µl; platelets ≥ 100 x 10³/µl; Hb ≥ 10 g/dl; absolute neutrophil count ≥ 1,5 x 10³/µl; bilirubin ≤ 2.0 mg/dl; AST, ALT and LDH less than 3 times upper limit of normal; serum creatinine ≤ 2 mg/dl;
- Fertile females have to practice adequate contraception;
- Signed informed consent;

3 OBJECTIVES

The main objective of this study is to evaluate the efficacy of a combined chemo-vaccine therapy in increasing the distant metastases-free survival of disease-free melanoma patients.

Primary endpoint.

Distant metastases-free survival (DMFS). Primary endpoint of the study is to determine whether DTIC plus vaccine administration could prevent distant relapse and death of patients with high-risk resected melanoma. DMFS is defined as the length of time from randomization to the first distant recurrence of melanoma. Patients will be monitored during study and site and interval of relapses were recorded. Treatment comparisons for DMFS will be conducted using a stratified log-rank test. The Kaplan-Meier method will be used to calculate plots of estimated relapse-free survival. Cox's proportional hazards regression will be used to assess the impact of treatment after adjustment for other patient characteristics.

Ferraresi/Cognetti IFO

Secondary endpoints

Magnitude and quality of the immune responses of treated patients and their correlations with clinical responses will be determined. The following immune parameters will be characterized.

- i) *DTH.* DTH test, by using vaccine peptides as antigens, will be performed as clinical measure of vaccine-induced immune response.
- ii) *CTL response.* The magnitude of the response will be determined by enumerating vaccine-specific CD8⁺ T cells in the peripheral blood. This will be carried out by ELISPOT as well as by tetramer staining assays. The quality of the response will be determined by measuring the lytic activity of CD8⁺ T cells, by standard ⁵¹Cr-release assay.

Nisticò IFO

Additional secondary end-points, determined only in patients who will give their consent, will be the followings.

- iii) *Cytokine multiparametric analysis.* The functional characterisation of vaccine-specific CD8⁺ T cells will be determined through the analysis of the production of a panel of cytokines measured by intracellular staining technique will be determined, and the correlation of these parameters with other immune as well as clinical responses will be evaluated.

Proietti ISS

- iv) *Gene expression profiles.* Microarray analysis will be also performed on RNA isolated from patients PBMCs before and one day after DTIC administration at selected time points, in order to possibly identify genes involved in DTIC-dependent activation of immune responses.

Aricò ISS

Studio 1: Importanza dell'analisi multiparametrica delle citochine

From www.bloodjournal.org at SWETS BLACKWELL INC on June 18, 2008. For personal use only.

IMMUNOBIOLOGY

HIV nonprogressors preferentially maintain highly functional HIV-specific CD8⁺ T cells

Michael R. Betts, Martha C. Nason, Sadie M. West, Stephen C. De Rosa, Stephen A. Migueles, Jonathan Abraham, Michael M. Lederman, Jose M. Benito, Paul A. Goepfert, Mark Connors, Mario Roederer, and Richard A. Koup

Establishing a CD8⁺ T cell-mediated immune correlate of protection in HIV disease is crucial to the development of vaccines designed to generate cell-mediated immunity. Historically, neither the quantity nor breadth of the HIV-specific CD8⁺ T-cell response has correlated conclusively with protection. **Here, we assess the quality of the HIV-specific CD8⁺ T-cell response by measuring 5 CD8⁺ T-cell functions (degranulation, IFN- γ , MIP-1 β , TNF- α , and IL-2) simultaneously in chronically HIV-infected individuals and**

elite nonprogressors. We find that the functional profile of HIV-specific CD8⁺ T cells in progressors is limited compared to that of nonprogressors, who consistently maintain highly functional CD8⁺ T cells. This limited functionality is independent of HLA type and T-cell memory phenotype, is HIV-specific rather than generalized, and is not effectively restored by therapeutic intervention. Whereas the total HIV-specific CD8⁺ T-cell frequency did not correlate with viral load, the frequency and proportion of the HIV-specific

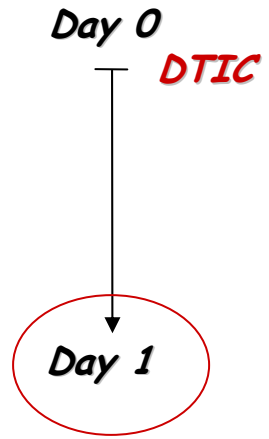
T-cell response with highest functionality inversely correlated with viral load in the progressors. Thus, rather than quantity or phenotype, the quality of the CD8⁺ T-cell functional response serves as an immune correlate of HIV disease progression and a potential qualifying factor for evaluation of HIV vaccine efficacy. (Blood. 2006;107:4781-4789)

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La messa a punto della metodica è stata avviata con successo

Microarray analysis of melanoma patients' PBMCs

(E. Aricò / F. Moschella)

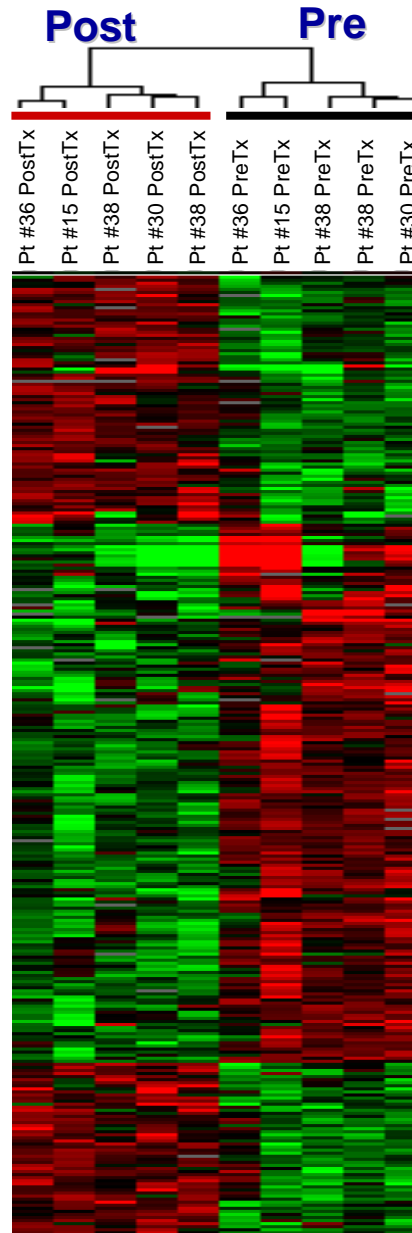


Gene Expression profiling

314 genes significantly differentially expressed ($P < 0.005$)

138 genes up-regulated
176 genes down-regulated

Down-regulated genes include genes involved in biosynthetic and metabolic processes



Biological Process of Up-regulated genes	Obs	Exp	Obs/Exp
Cytokine production IL6 SYK LCP2 TLR2	4	0.7	5.4
Leukocyte activation IL6 SYK LCP2 TNFSF13	4	1.4	2.9
Immune response BST1 IFIT2 LILRA4 CD97 IL15 SYK CLEC4A IL6 TLR2 CXCL10 LCP2 TNFSF13 CXCL3	13	4.5	2.9
Cell motility C3AR1 CXCL16 STAT3 CD97 PLAUR SYK CXCL10 SPINT2	8	3.1	2.6
Generation of precursor metabolites and energy ATP5B CYP27A1 IBRDC2 ATP6V0B GAK INSR ATP6V0D1 GPD2 PYGL COX5B SYK ZAK IL15	10	3.9	2.6
Response to external stimuli AIF1 CXCL16 STAT3 C3AR1 CXCL3 SYK CD97 IL6 TLR2 CXCL10 PLAUR	11	4.5	2.4
Defense response AIF1 CXCL10 STAT3 C3AR1 CXCL3 TCIRG1 CD97 IL6 TLR2	9	3.8	2.4
Circulation C3AR1 CXCL10 SOAT1	3	1.3	2.3

Studio 1: definizione dello schema dei prelievi

Blood collection

ARM I	ARM II
Pre Tx - 70 ml of peripheral blood	
<ul style="list-style-type: none"> - 6.5 ml hemato-bio-chemistry analysis - 3.5 ml autoimmunity analysis - 30 ml FMT - 10 ml HLA/A2 tetramer staining - 10 ml IFN-γ ELISPOT assay - 10 ml gene profiling analysis 	<ul style="list-style-type: none"> - 6.5 ml hemato-bio-chemistry analysis - 3.5 ml autoimmunity analysis - 30 ml FMT - 10 ml HLA/A2 tetramer staining - 10 ml IFN-γ ELISPOT assay - 10 ml gene profiling analysis
T0 - 60 ml of peripheral blood	
	<ul style="list-style-type: none"> - 3 ml hemochrome - 20 ml HLA/A2 tetramer staining - 20 ml IFN-γ ELISPOT assay - 17 ml NKG2D study
T1 - 70 ml of peripheral blood	
<ul style="list-style-type: none"> - 3 ml hemochrome - 20 ml HLA/A2 tetramer staining - 20 ml IFN-γ ELISPOT assay - 17 ml NKG2D study - 10 ml gene profiling analysis 	<ul style="list-style-type: none"> - 10 ml gene profiling analysis
T2 - 10 ml of peripheral blood	
<ul style="list-style-type: none"> - 10 ml gene profiling analysis 	<ul style="list-style-type: none"> - 10 ml gene profiling analysis
T8 - 10 ml of peripheral blood	
<ul style="list-style-type: none"> - 10 ml gene profiling analysis 	<ul style="list-style-type: none"> - 10 ml gene profiling analysis
T21 - 70 ml of peripheral blood	
	<ul style="list-style-type: none"> - 3 ml hemochrome - 22 ml HLA/A2 tetramer staining - 22 ml IFN-γ ELISPOT assay - 23 ml NKG2D study
T22 - 70 ml of peripheral blood	
<ul style="list-style-type: none"> - 3 ml hemochrome - 22 ml HLA/A2 tetramer staining - 22 ml IFN-γ ELISPOT assay - 23 ml NKG2D study 	
T29 - 20 ml of peripheral blood	
<ul style="list-style-type: none"> - 20 ml NKG2D study 	<ul style="list-style-type: none"> - 20 ml NKG2D study
T42 - 70 ml of peripheral blood	
	<ul style="list-style-type: none"> - 3 ml hemochrome - 22 ml HLA/A2 tetramer staining - 22 ml IFN-γ ELISPOT assay - 23 ml NKG2D study
T43 - 70 ml of peripheral blood	
<ul style="list-style-type: none"> - 3 ml hemochrome - 22 ml HLA/A2 tetramer staining - 22 ml IFN-γ ELISPOT assay - 23 ml NKG2D study 	
T63 - 70 ml of peripheral blood	

Studio 1: preparazione di CRF cartacee (I. Capone / F. Urbani) e elettroniche (in collaborazione con Claudio Di Benedetto ISS)

ISS-DPII	N° Paziente	Iniziali Paziente	Visita Pre-Tx	N° Pag 1

DICHIARAZIONE DI ACCETTAZIONE DEL CONSENSO INFORMATO		DATA		
		giorno	mese	anno
Il sottoscritto _____				
attesta che il paziente N° [] [] [] [] le cui iniziali sono [] [] [] []				
ha dato il suo consenso a partecipare allo studio dopo aver letto e firmato il foglio di consenso informato.				
Data in cui è stato ottenuto il consenso [] [] [] [] [] [] [] [] [] [] [] []				
giorno mese anno				
Firma del medico responsabile _____				

DATI DEMOGRAFICI											
DATA DI NASCITA				SESSO				RAZZA			
				M			F	Caucasica			
								Nera			
								Orientale			
								Asiatica			
								Altra, specificare:			
giorno	mese	anno									

ISS-DPII	N° Paziente	Iniziali Paziente	Visita Pre-Tx	N° Pag 2

ANAMNESI					
Il paziente riferisce una patologia attuale o pregressa a carico di uno dei seguenti sistemi?					
Sistema	SI	No	Se sì, specificare la diagnosi e la data di insorgenza (se conosciuta)	Indicare se ancora in corso	
				SI	No
Cardiovascolare					
Respiratorio					
Osteoarticolare					
Occhio, naso, gola					
Epatobiliare					
Endocrino					
Ematopoietico					
Nervoso Centrale					
Dermatologico					
Genito-Urinario					
Gastrointestinale					
Malattie Psichiatriche					
Allergie					
Altro (specificare)					

TERAPIA PRECEDENTE E CONCOMITANTE				<i>In caso di necessità questa sezione può essere moltiplicata</i>	
Il paziente è o è stato sottoposto a terapia?				SI	No
Nome del farmaco	Data				
	giorno mese anno				
	inizio				
	fine				
in corso					

Studio 1: Il protocollo clinico è in corso di sottomissione al comitato etico dell' Istituto Regina Elena per la cura dei tumori (IFO Roma)

Studi preclinici avviati

Angela Battistini, ISS, Dip. Malattie Infettive, Patogenesi Molecolare, ISS.

Ruolo del fattore di trascrizione IRF1 sulla modulazione dell'attività Treg indotta dal trattamento chemioterapico.

Margherita Bignami, Cancerogenesi Sper., Dip. Ambiente e Prev. Primaria, ISS.

Studio del ruolo dei sistemi di riparazione del danno al DNA indotto dai chemioterapici sull'induzione dei meccanismi omeostatici del sistema linfo emopoietico

Franca Podo, Dip. Farmaco, Farmacogenetica e Farmacoresistenza, ISS.

Analisi in vitro e in vivo, mediante spettroscopia di risonanza magnetica, delle variazioni di pH intratumorale indotte da inibitori delle pompe protoniche e del loro effetto sulla risposta immune antitumorale

Federica Moschella, Dip. Biologia Cellulare e Neuroscienze, ISS.

Analisi comparativa, mediante microarray, dell'espressione genica di cellule del sistema linfo emopoietico dopo trattamento con diversi chemioterapici antitumorali.

Studi in fase di preparazione

Studio di vaccinazione di pazienti affetti da melanoma con cellule dendritiche autologhe caricate con corpi apoptotici del tumore stesso e reinfuse dopo trattamento con dacarbazine.

Lo studio sarà eseguito in pazienti con melanoma in stadio III e IV in cui il tumore è stato rimosso chirurgicamente. Il tessuto tumorale verrà in parte utilizzato per la caratterizzazione istologica e immunohistochimica e, in parte per la produzione di corpi apoptotici da usare come antigeni. I corpi apoptotici saranno caricati su cellule dendritiche ottenute dai monociti del sangue e coltivate in presenza di GM-CSF e IFN-alfa in condizioni GMP (IFN-DC).

Questo studio si avvarrà delle tecniche di monitoraggio clinico e immunologico messe a punto nello studio precedente

Questo studio si avvarrà della struttura FaBioCell (ISS) per la preparazione delle DC e dei corpi apoptotici da tumore autologo (Rozera/Ferrantini)