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CAR-T and immunomonitoring: methodological perspectives

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IRCCS Ospedale San Raffaele, Milano

IV Congresso Nazionale GIIMA
Roma, 29-11-2019

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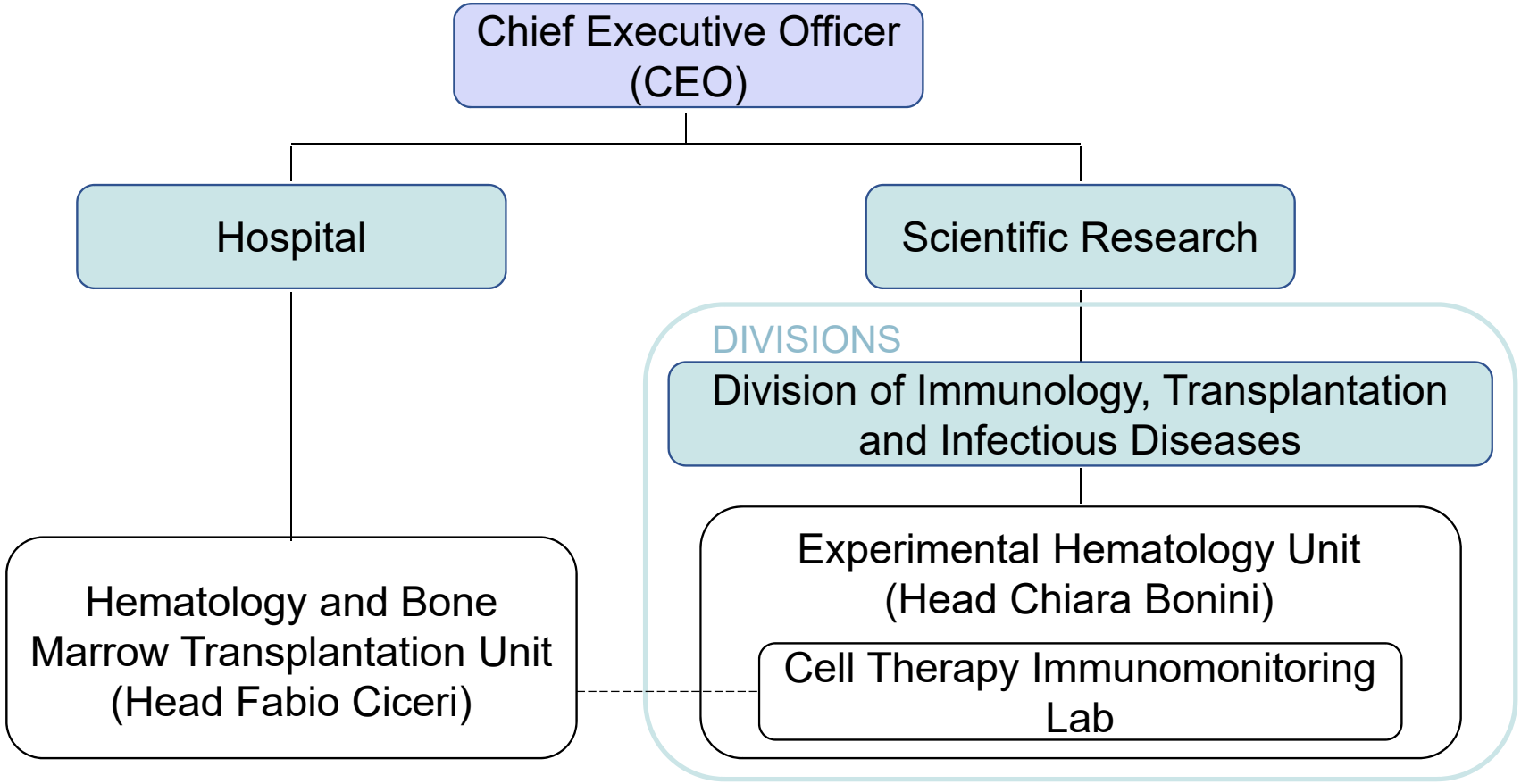
Cell Therapy Immunomonitoring Lab (MITiCi) organizational chart



MISSION: To monitor immune-related events in clinical trials, with the final aim of providing additional information to tailor patient management and treatment

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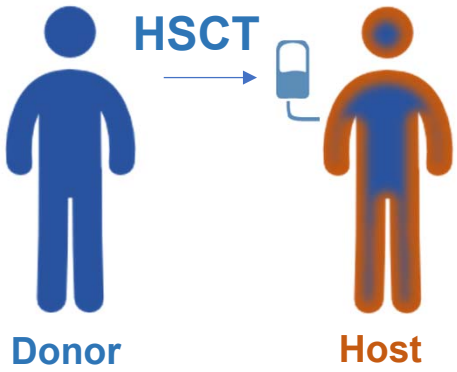
Identification and validation of immune biomarkers able to predict clinical outcome after HSCT

Relapse Incidence

- Tumor-specific T cells are exhausted in patients prone to relapse
- Early differentiated T_{CM} and T_{SCM} are exhausted at relapse (*Noviello, Manfredi et al., Nat Comm 2019*)

Non Relapse Mortality

Higher $CD8^+$ T cell counts predict better haplo-HSCT outcome (*Bondanza et al, BMT 2018*)



GvHD

Sirolimus-based GvHD prophylaxis promotes the in vivo expansion of T_{REG} (*Peccatori et al., Leukemia 2015*) (*Cieri et al., BBMT 2015*)

Severe Infectious adverse events

Early recovery of CMV immunity after HSCT predicts the risk of infectious complications (*Noviello et al., BMT 2015*)

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Immunomonitoring in cell- and gene-therapy

Tracking genetically engineered lymphocytes long-term reveals the dynamics of T cell immunological memory

Giacomo Oliveira,[1,2] Eliana Ruggiero,[1,3] Maria Teresa Lupo Stanghellini,[4] Nicoletta Cieri,[1]* Mattio D'Agostino,[1,2] Raffaele Fronza,[3] Christina Lulay,[3] Francesca Dionisio,[5] Sara Mastaglio,[1,4] Raffaella Greco,[4] Jacopo Peccatori,[4] Alessandro Aiuti,[5] Alessandro Ambrosi,[2] Luca Biasco,[5] Attilio Bondanza,[4,6] Antonio Lambiase,[7] Catia Traversari,[7] Luca Vago,[4,8] Christof von Kalle,[3] Manfred Schmidt,[3] Claudio Bordignon,[2,7] Fabio Ciceri,[2,4,8] Chiara Bonini[1,2†]

www.ScienceTranslationalMedicine.org 9 December 2015 Vol 7 Issue 317 317ra198

EMBO Molecular Medicine

Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne muscular dystrophy

Giulio Cossu[1,*], Stefano C Previtali[2,3,**], Sara Napolitano[4,5], Maria Pia Cicalese[4,5], Francesco Saverio Tedesco[6], Francesca Nicastro[7,8], Maddalena Noviello[9], Urmas Roostalu[1], Maria Grazia Natali Sora[3], Marina Scarlato[3], Maurizio De Pellegrin[10], Claudia Godi[8,11], Serena Giuliani[5], Francesca Ciotti[5], Rossana Tonlorenzi[2], Isabella Lorenzetti[2], Cristina Rivellini[2], Sara Benedetti[6], Roberto Gatti[7], Sarah Marktel[5], Benedetta Mazzi[12], Andrea Tettamanti[7], Martina Ragazzi[6], Maria Adele Imro[13], Giuseppina Marano[13], Alessandro Ambrosi[14], Rossana Fiori[15], Maria Pia Sormani[16], Chiara Bonini[9], Massimo Venturini[17], Letterio S Politi[11], Yvan Torrente[8,***] & Fabio Ciceri[4,****]

EMBO Mol Med (2015)7:1513-1528

nature medicine

Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells

Fulvio Mavilio[1], Graziella Pellegrini[1,2], Stefano Ferrari[2], Francesca Di Nunzio[1], Enzo Di Iorio[2], Alessandra Recchia[1], Giulietta Maruggi[1], Giuliana Ferrari[3], Elena Provasi[4], Chiara Bonini[4], Sergio Capurro[5], Andrea Conti[6], Cristina Magnoni[6], Alberto Giannetti[6] & Michele De Luca[1,2]

NATURE MEDICINE VOLUME 12 | NUMBER 12 | DECEMBER 2006

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To perform immunomonitoring of phase I clinical trials we need to comply with GCLP standard (Determina AIFA 809/2015)

SCOPE:

To provide a framework for the analysis of samples from clinical trials to ensure the reliability, consistency and integrity of the data generated while protecting the rights, safety and well-being of participants

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To perform immunomonitoring of phase I clinical trials we need to comply with GCLP standard (Determina AIFA 809/2015)

LEGAL REQUIREMENTS:

World Health Organization

Good Clinical Laboratory Practice (GCLP)

ISO 9001:2015

Italian Medicines Agency

GCP internal Audit
4th September 2019

Start date:
July 2018

ISO certification
13th May 2019

AIFA self-certification
25th October 2019

AIFA authorization
17th November

Quality System certification and AIFA authorization process

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Requirements of the international guidelines

ISO:

1. Mission of the Lab and context analysis

2. Implementation of Project planning tools and risks analysis approach

3. Continuous improvement: identification of goals and control of the performance

GCLP:

4. Ensure that qualified **personnel**, appropriate **facilities**, **equipment** and **materials** are available in the trial facility

5. Procedures for the receipt, handling, storage, retrieval and management of **trial materials** should be designed to prevent mix-ups and maintain their integrity

6. A written **analytical plan** should exist prior to the initiation of the work and be available to all the staff

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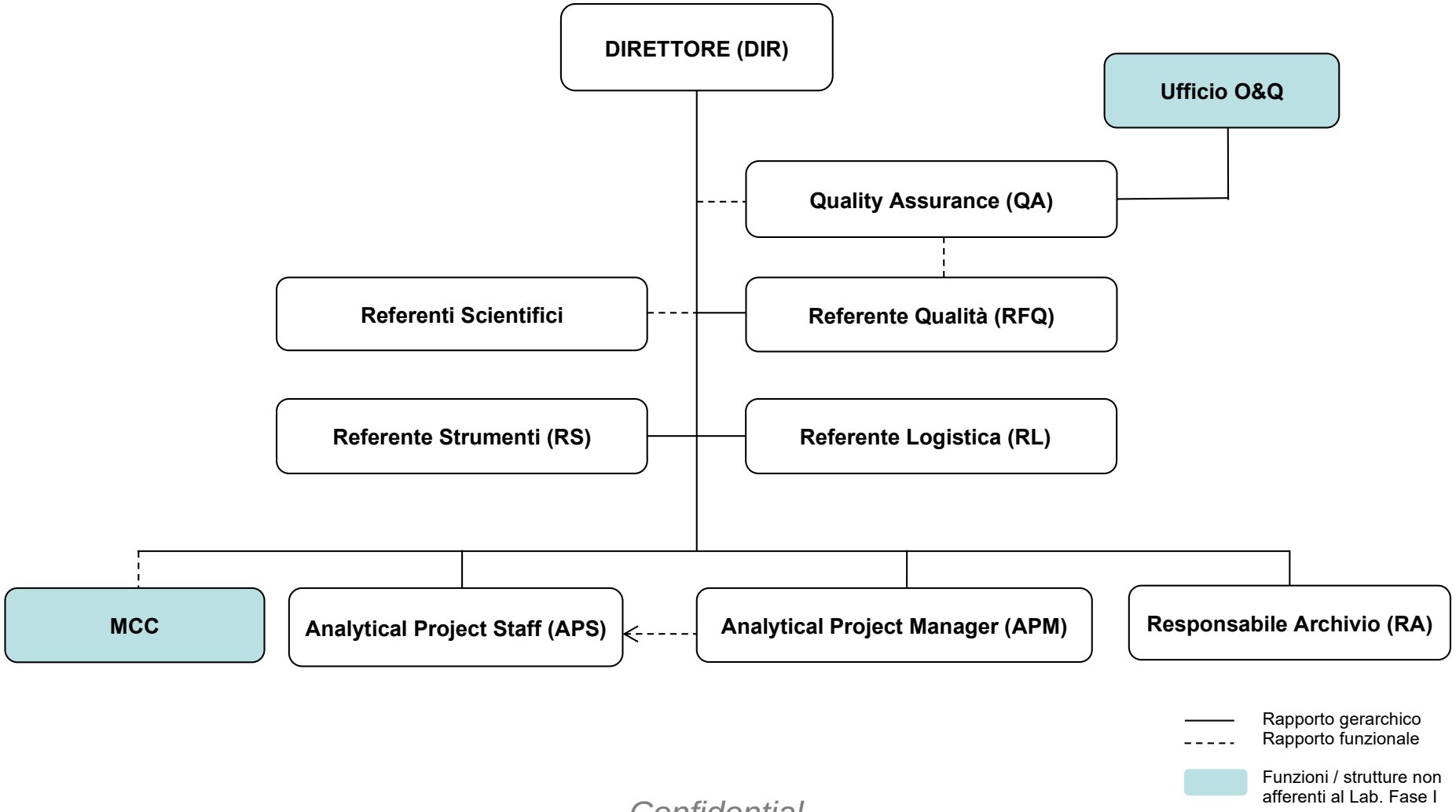
Organization and personnel



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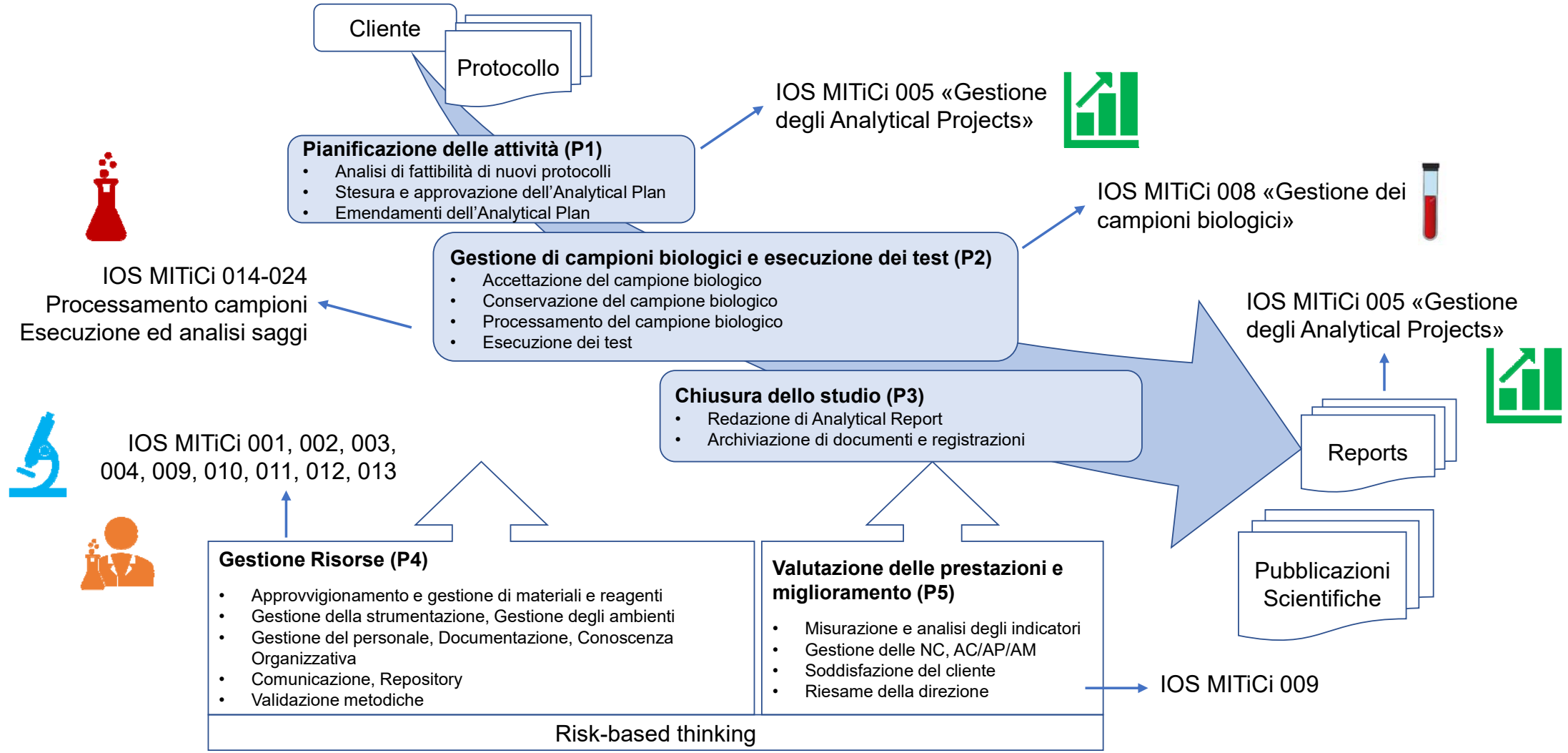
Project planning tools: our process map



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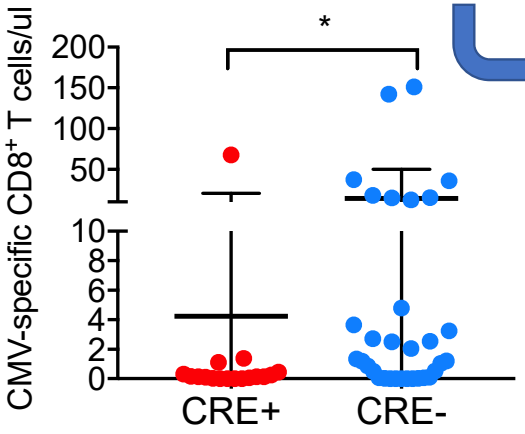
Prospective observational study to evaluate the relevance of CMV- specific T-cell frequencies in patients undergoing allogeneic HSCT

45 days after HSCT



CRE, CMV Clinically Relevant Events

75 patients enrolled

10 timepoints pe
patient up to 1 y
after HSCT

630
proces
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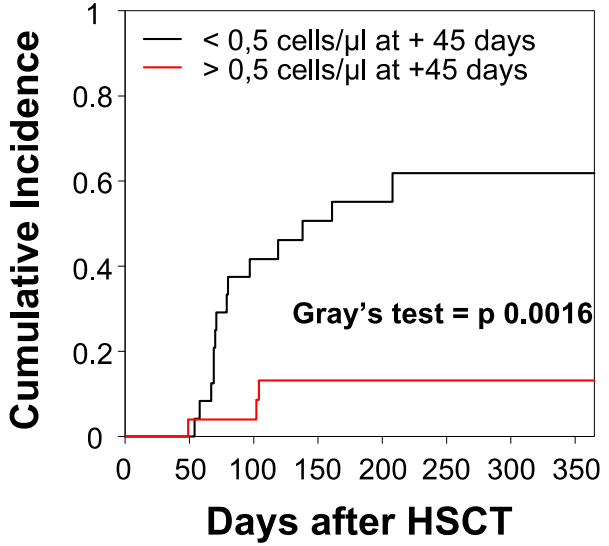
CMV Clinically Relevant Events (CRE)



Patients at Risk									
< 0,5	24	24	15	11	9	6	6	4
> 0,5	25	25	21	17	7	6	6	5

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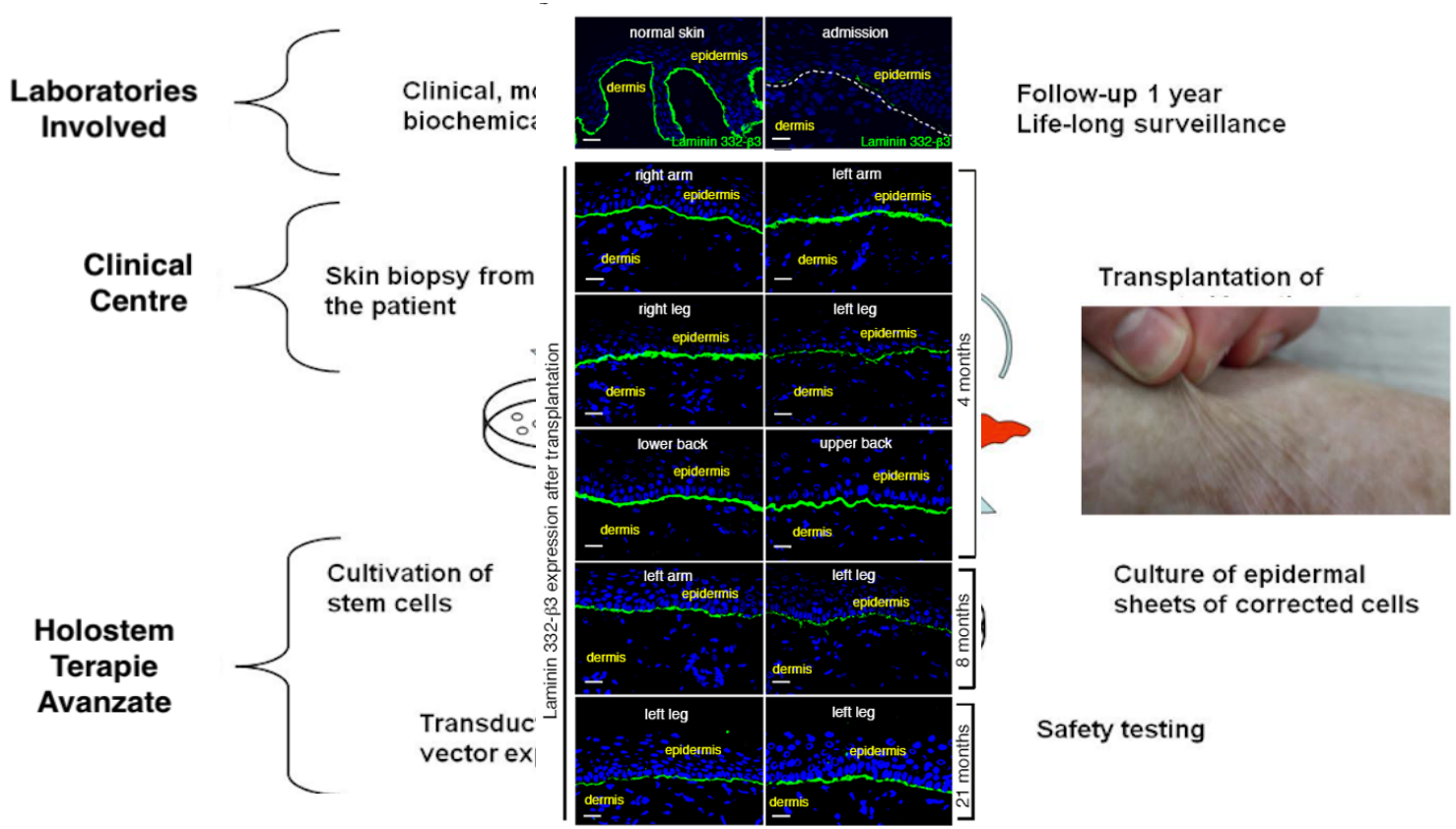
Phase II clinical trial to confirm the efficacy and safety of autologous genetically modified epidermal grafts in patients with junctional Epidermolysis Bullosa



Hirsch et al, Nature 2017

Sponsor: Holostem

Secondary Endpoints

…

To evaluate the potential immune reactions against the transgenic protein

What we'll do

Longitudinal analysis before and after therapy to evaluate the presence of potentially harmful immune responses against Laminin 332

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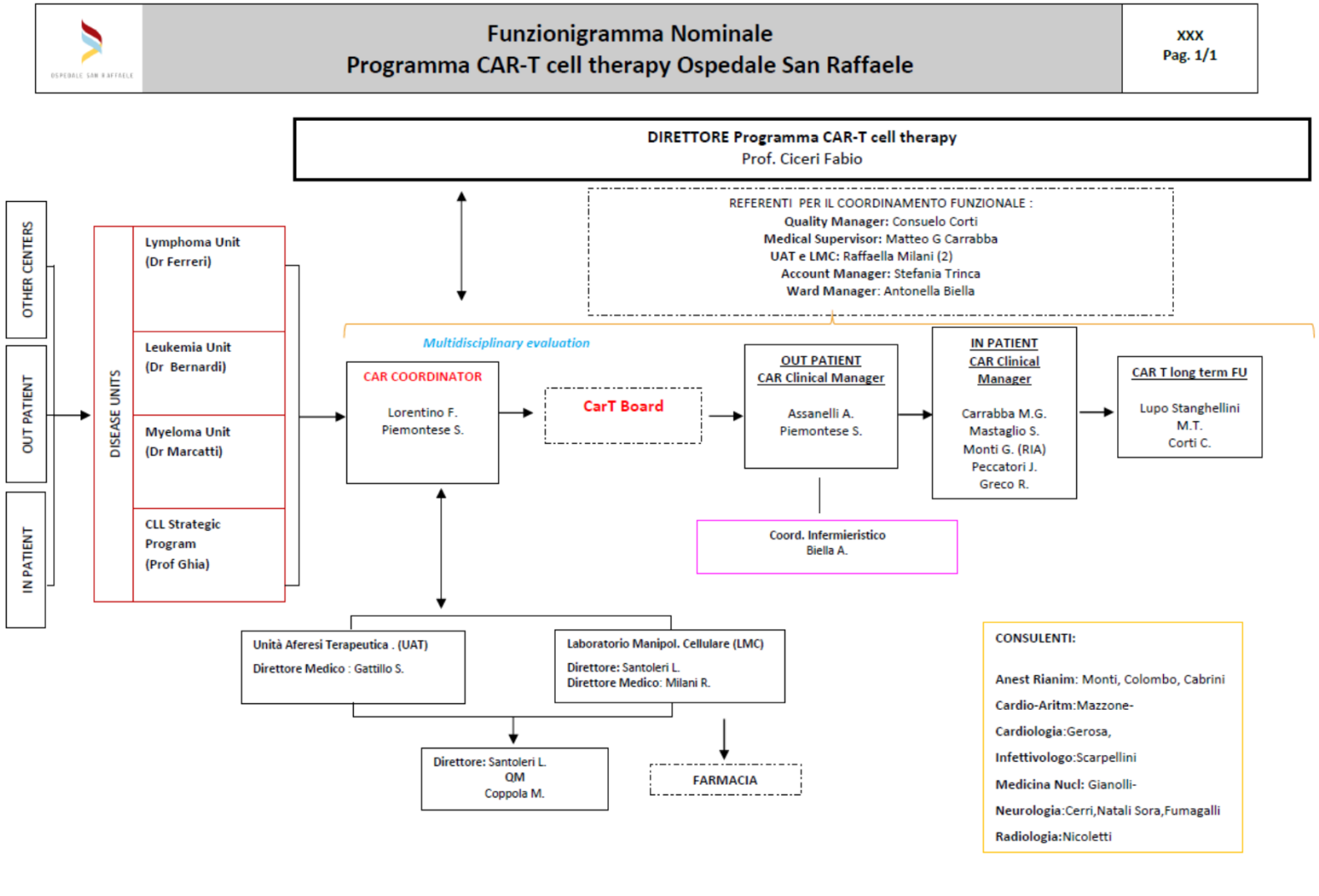
Clinical CAR-T cell therapy program in OSR



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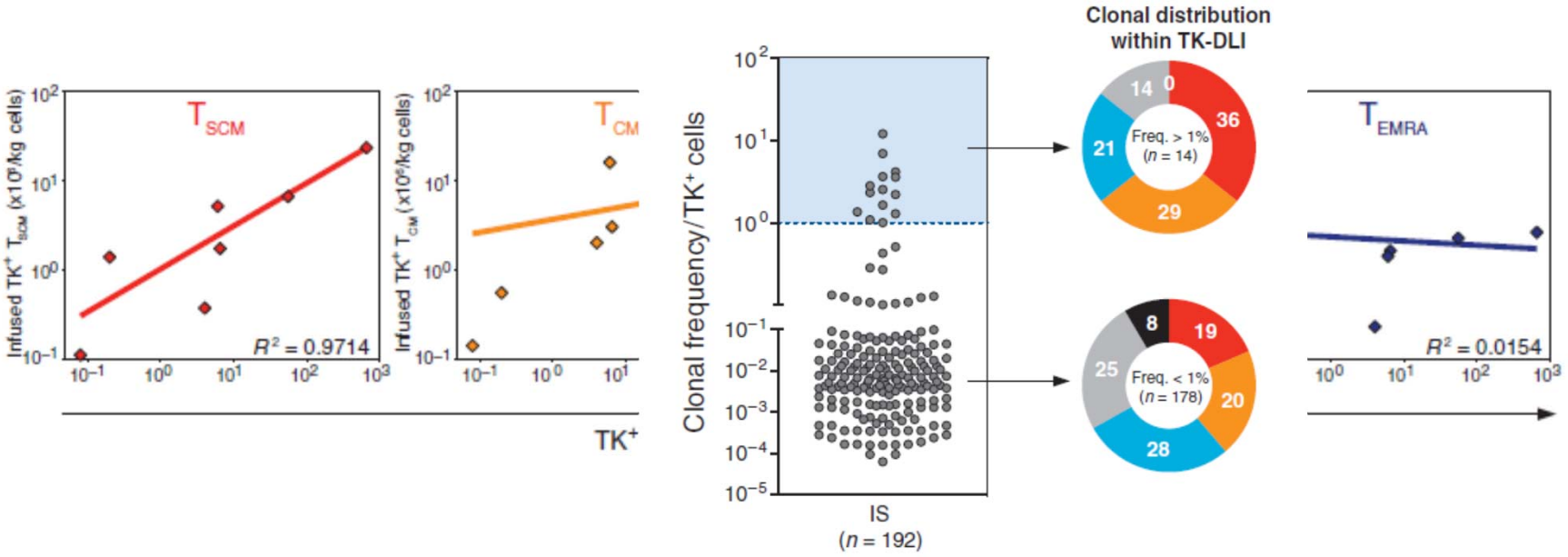
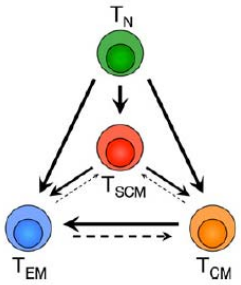
Suicide gene therapy after HSCT: immune correlates to persistence

Long-term persistence of genetically modified cells correlates with the amount of infused T_{SCM} and is mediated by antigen exposure (Oliveira Sci Transl Med 2015)



Oliveira et al,
Sci Transl Med 2015



Cieri et al,
Blood 2015

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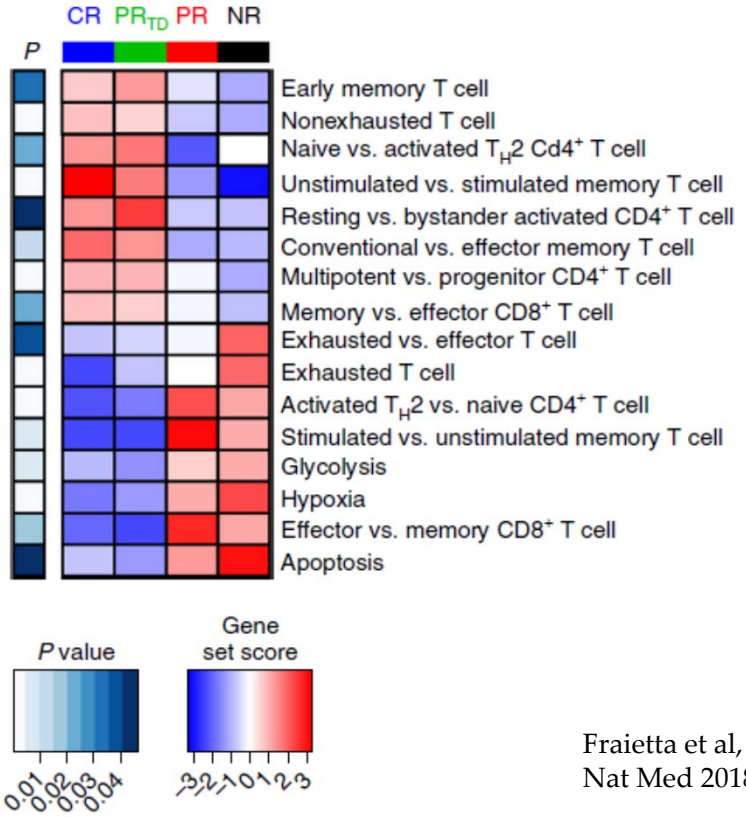
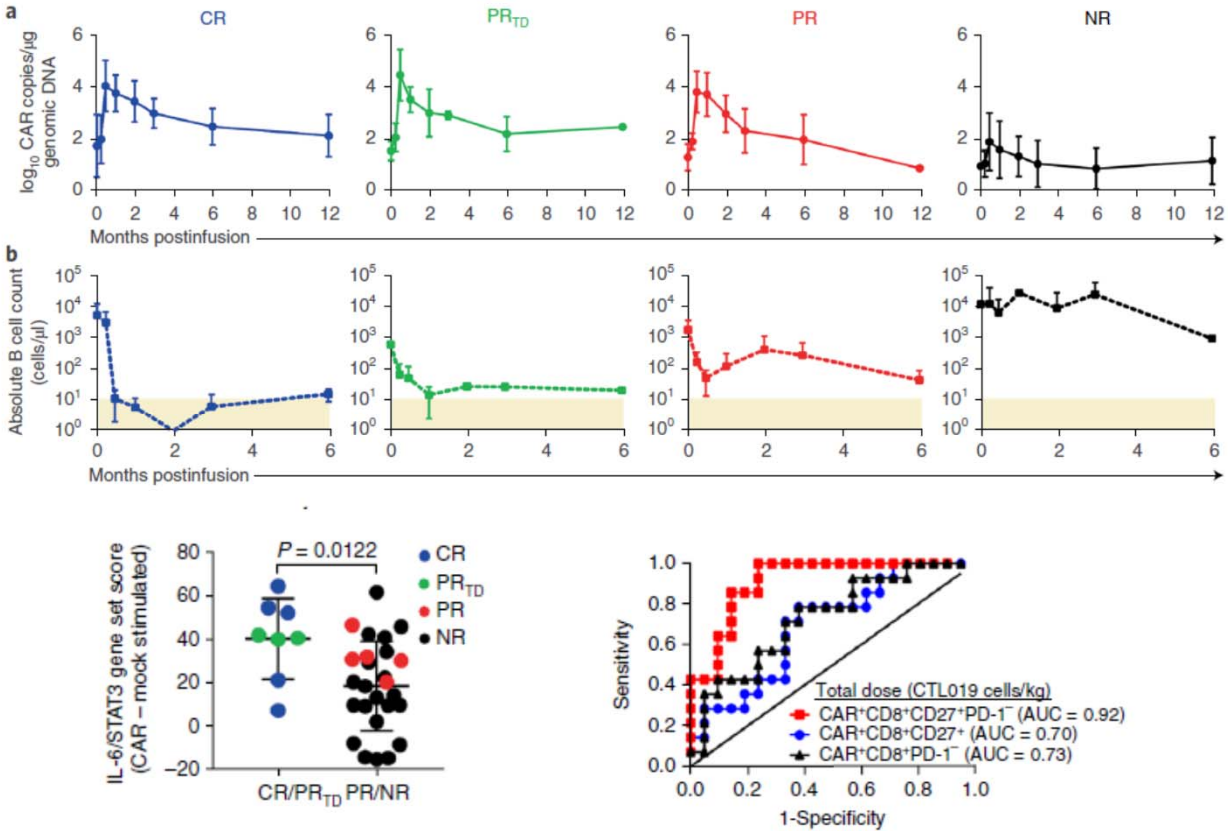
CAR-T: immune correlates to response

Response to CAR-T is correlated to composition of T cell products, CAR-T expansion and persistence, presence of B cell aplasia, IL-6/STAT3 pathway activation and percentage of $CD8^+CD27^+PD\text{-}1^-$ in CAR-T (Porter Sci Transl Med 2015, Fraietta Nat Med 2018)



Fraietta et al, Nat Med 2018



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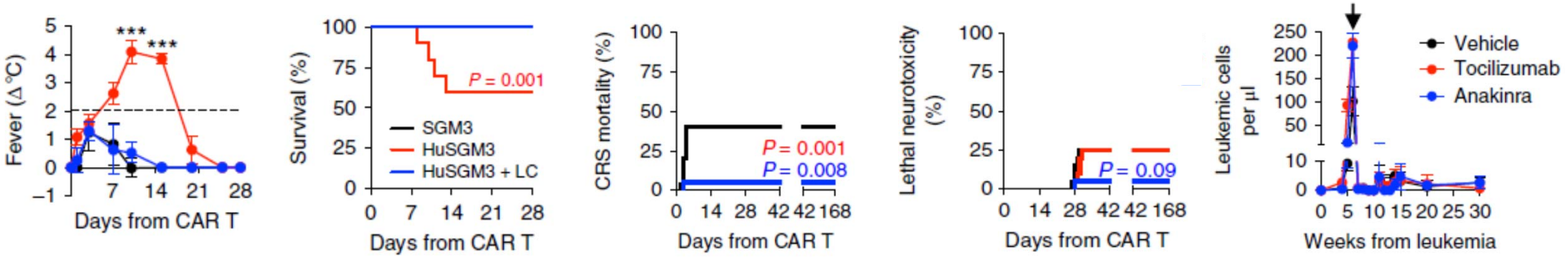
CAR-T: immune correlates to toxicity

CAR-T induced CRS and neurotoxicity in preclinical models are mediated by myeloid cells-derived IL-6, IL-1 and iNOS (Norelli Nat Med 2018, Giavridis Nat Med 2018)



Norelli et al,
Nat Med 2018

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Immunomonitoring in CAR-T: our plan

- ✓ To perform a longitudinal detailed analysis of T and B lymphocytes and myeloid cells, including presence of $CD8^{+}CD27^{+}$PD-1-, TSCM phenotype and expression of molecules involved in myeloid-lymphoid interactions
- ✓ To specifically evaluate CAR-T fitness and correlate it to response to therapy
- ✓ To dissect the contribution of myeloid-, endothelial- and T cell- derived factors to the development of severe CRS and neurotoxicity
- ✓ To trace CAR-T clonotypes by molecular markers

→ Immune correlates to response, long-term persistence and toxicity

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Thanks to

Cell Therapy Immunomonitoring Lab (MITiCi)

Chiara Bonini
Pantaleo De Simone
Maddalena Noviello

Valeria Beretta
Monica Casucci
Laura Falcone
Lucia Lopalco
Claudia Pastori

Organization and Quality Management Office – Research Area
Vanda Parezanovic
Vincenza Maselli
Elisabetta Zino

Hematology and BMT Unit
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Matteo Carrabba
Raffaella Greco
Maria Teresa Lupo-Stanghellini
Sara Mastaglio
Jacopo Peccatori

Study coordination and data management
Stefania Trinca
Daniela Piazzolla
Massimiliano Vicini

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Biobank
Cristina Tresoldi
Elisa Cantarelli
Michela Grossi
Serenesse Tomasi

Experimental Hematology Unit
Chiara Bonini
all the lab

Holostem Terapie Avanzate, Modena
Michele De Luca
Giada De Leo
Laura Di Rosa