OSPEDALE
SAN RAFFAELE

AVAILABLE TECHNOLOGIES

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INDEX

Cancer

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Sector
Cancer

Development stage
Preclinical proof of concept

Key publications
[1]Casucci et al. Blood. 2013
[2]Greco et al. Sci Trans Med. 2022.
[3]Kaneko et al. Blood. 2009.
[4]Arcangeli et al. JCI. 2022.
[5]Raccosta et al. Cell Death & Dis. 2023.
[6]Provasi et al. Nat Med. 2012.
[7]Ruggiero et al. Sci Trans Med. 2022.

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

Adoptive cell therapy program

CLINICAL NEED

Adoptive T cell therapy (ACT) has become a promising option for cancer patients. Tumor-infiltrating lymphocytes and T cells genetically redirected to the tumor by T cell receptor (TCR), or chimeric antigen receptor (CAR) gene transfer are in clinical validation against a variety of malignancies. Different institutions have recently reported impressive clinical responses by infusing CD19 CAR T cells in patients with B-cell acute lymphoblastic leukemia (ALL) or B-cell lymphoma. However, there are increasing reports of subjects with ALL who relapsed following CD19 CAR therapy due to the loss of CD19 expression on leukemic cells (30-60%¥). In addition, first-in-man studies revealed unique hurdles contributing to the lack of a sharp demonstration of efficacy of CAR T cells in the context of solid malignancies.

SOLUTION & BENEFITS

To overcome current hurdles faced by adoptive cell therapy approaches, with respect to both hematological and solid tumors, we developed the below pipeline with the aim to:

- increase efficacy in solid tumors;
- improve fitness and performance of T cell products;
- overcome limitations of current ACT;
- allow future allogeneic T cell therapies.

Indeed, we identified new CAR and TCR specificities for both hematological and solid tumors and developed 'enabling' strategies to improve their antitumor efficacy through several mechanisms: (i) unmasking of tumor antigens and potentiating immunological synapse (de-glycosylation); (ii) reduction of inhibitory receptor engagement (de-glycosylation and inhibitory receptor disruption); (iii) improvement of engineered T cell fitness (optimized T cell selection and culture methods), and (iv) production of allogeneic T cell products (ZINC-finger nucleases- and CRISPR-based approaches).

PRODUCT & TECHNOLOGY

Category	Technology	Field/Application	Development Stage
CAR	CD44v6	Solid tumors (GI, ovarian and bladder)	In vivo PoC[1,2]
	CDH17	Solid tumors (gastrointestinal tumors, liver metastasis)	Lead opt./ Animal test.
	Undisclosed target		
TCR	hTert	Hematological malignancies and solid tumors	Lead opt./ Animal test.
	Survivin		
Enablers	2-deoxy-D-glucose	Combination with any CAR T	In vivo PoC[2]
	PNGase	Combination with any CAR T	Lead characterization
Methods	IL7/IL15	Manufacturing of engineered T cells highly enriched of stem cell memory T cells and characterized by improved fitness and safety	GMP-grade reagents[3]
	Tn/Tscm preselection		In vivo PoC[4]
	LXR antagonists		In vivo PoC[5]
	ZINC-finger nucleases for TCR disruption	Combination with any TCR/CAR	In vivo PoC[6]
	CRISPR-based inhibitory receptor and/or TCR disruption		In vivo PoC[7]

MARKET & COMPETITION

The global market for adoptive cell therapy applied to both hematological and solid tumors in the last three years is made up of 10.138 deals for an overall value of 1.1 trillion USD[¥¥].

[¥]Xu et al. Front Immunol. 2019.
[¥¥]GlobalData, May 2022.

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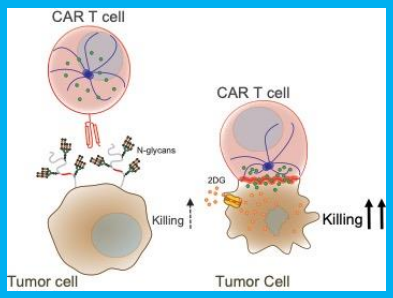
Sector
Cancer

Key Publication
Greco et al., Sci. Transl. Med. 2022.

Development stage
Preclinical Development

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

The Technology



I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

Inhibition of glycosylation to potentiate CAR-T cell therapy

CLINICAL NEED

Chimeric antigen receptors (CARs) are artificial molecules combining antigen-binding and T-cell activating functions into a single receptor, to provide T cells with the ability to target a desired antigen. Most commonly, the antigen-binding moiety is represented by the single-chain fragment variable of a monoclonal antibody. T cells engineered to express a tumor-specific CAR (CAR T cells) represent a great promise in the context of cancer immunotherapy. Different institutions have recently reported impressive clinical responses by infusing CD19 CAR T cells in patients with refractory B-cell malignancies. However, first-in-man studies revealed unique hurdles contributing to the lack of a sharp demonstration of efficacy of CAR T cells in the context of solid malignancies. The first requirement for CAR T cells to work properly is efficient antigen engagement, which leads to the formation of an immunological synapse able to activate effector functions and drive tumor cell elimination. Glycosylation is the enzymatic process linking sugars to other sugars, proteins or lipids. It has been shown that glycosylation alterations are very frequent in cancer, where they promote tumor growth and metastasis. These changes comprise increased branching of N-glycans that form huge sugar structures on the surface of malignant cells. Interestingly, it has been reported that these glycans can mask peptidic epitopes from antibody recognition and are required for the proper interaction of co-inhibitory ligand/receptor pairs that drive T-cell exhaustion.

PRODUCT & TECHNOLOGY

We have shown that an excess of branched N-glycans is present on multiple carcinomas and that these sugar structures inhibit the killing of malignant cells by CAR T cells. The invention regards the exploitation of glycosylation inhibition to improve the efficacy of CAR T cells in solid tumors. In this context, we report that tumor treatment with 2-Deoxy-D-glucose (2DG), a de-glycosylating agent, causes membrane exposure of de-glycosylated antigens, sensitizing tumor cells to be recognized and killed by CAR T cells. Moreover, we have shown that glycosylation inhibition ameliorates the exhaustion profile of CAR T cells and preserve their fitness in vivo. The efficacy of combining CAR T cell therapy with 2DG administration has been demonstrated with different CAR specificities and multiple types of solid malignancies.

SOLUTION & BENEFITS

2-DG improves CAR T-cell efficacy through several mechanisms: (i) unmasks tumor antigens; (ii) potentiates immunological synapse; (iii) reduces inhibitory receptors' engagement; (iv) improves CAR T cells' fitness, (v) can kill hypoxic tumors, (vi) exerts a beneficial effect on the tumor microenvironment (TME). 2-DG is safe due to its preferential accumulation in tumor cells. It has been extensively used in clinical trials (>200 patients), where it showed good tolerability with only minimal and reversible side effects (hypoglycemic symptoms).

MARKET & COMPETITION

The global market regarding CAR T-cell therapy applied to only solid tumors is made up of 6.227 past and ongoing deals for an overall value of billions of dollars. There are many competitors on the market considering all combinatorial treatments with the aim to potentiate CAR T cells efficacy, such as small molecules, gene therapy, and monoclonal antibodies. However, to the best of our knowledge, this project is the only inhibiting N-glycosylation to improve CAR-T efficacy against cancers. The added value of this technology is that it can be applied to multiple tumors and CARs, does not require new CAR design, and allows overcoming several barriers at once as such defective antigen engagement and tumor cell killing, T-cell exhaustion, T-cell differentiation, Immunosuppressive TME.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Combination of a glycosylation inhibitor with one CAR cell therapy for treating cancer	WO2020020841	Pending	Europe, USA, Canada, China, Japan, Australia and Israel

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Sector
Cancer

Key Publications
In preparation

Development stage
Preclinical Development

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

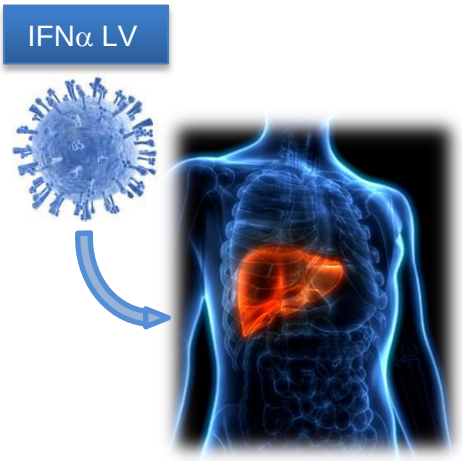
The technology
A novel lentiviral vector platform to selectively engineer liver resident macrophages to deliver therapeutic transgenes or tumor vaccines.

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

Liver-targeted delivery of therapeutic transgenes or tumor vaccines for liver cancer eradication

CLINICAL NEED

The liver is one of the most common sites for cancer metastasis, accounting for nearly 25% of all cases. The most common metastases originated from colorectal primaries (20-25%), followed by pancreatic and breast. The immune suppressive environment of the liver as well as its vascular architecture favor metastatic colonization from gastrointestinal tumors, leading to reduced patient survival and poor response to current pharmacological treatments, including immunotherapy. The dual blood supply of the liver (portal vein and the hepatic artery) not only makes it uniquely susceptible to metastasis from gastrointestinal cancers but also accessible to interventional therapies. Therefore, harnessing new interventional tools is imperative.



SOLUTION and BENEFITS

The technology developed by Dr. Squadrito is a novel lentiviral vector (LV) platform to selectively engineer liver resident macrophages (Kupffer cells) to deliver therapeutic transgenes (e.g. cytokines) or tumor antigens specifically to liver. In vivo studies using IFNa as therapeutic transgene, demonstrated robust and stable expression of the transgene by transduced Kupffer cells with no toxicity. IFNα induced substantial reprogramming of tumor microenvironment towards an immune activating state. Therapeutic efficacy has been shown in relevant mouse models of colorectal cancer liver metastasis. This technology provides a new therapeutic strategy with feasible translatability to the clinic for the treatment of cancer patients.

Key features

- Sustained transgene expression
- Off-the-shelf cancer gene therapy
- Single dose
- Compatible with not integrating LVs

Highly selective

- Liver targeting by VSV-LVs
- Macrophage specific promoter
- miRNA regulated expression of transgene

Safety

- No hepatic toxicity
- No autoreactive antibodies
- No histopathologic abnormalities

PRECLINICAL RESULTS

Use of this platform on distinct mouse models of colorectal and pancreatic ductal adenocarcinoma liver metastases significantly delayed tumor growth. Anti-tumor response was associated with activation of tumor-associated macrophages (TAM), enhanced MHCII-restricted antigen presentation by tumor-infiltrating dendritic cells and reduced exhaustion of CD8+ T cells.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Kupffer Cell-Targeting Lentiviral Vector	WO/2022/117876	Pending	PCT

Side-chain modified ergosterol and stigmasterol derivatives as Liver X Receptor (LXR) modulators

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Sector
Cancer

Key Publications
Marinozzi M. *et al.*, J. Med. Chem. 2017.

Development stage
Preclinical Development

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

The Technology
Portfolio of 16 novel steroidal stigmasterol and ergosterol derivatives that function as Liver X Receptor (LXR) agonists.

CLINICAL NEED

The past two decades have seen an exponential increase in the number of studies on the physiological roles of mammalian oxysterols, as well as on their contribution to the pathogenesis of different diseases. The breakthrough was the identification of a specific subset of oxysterols as endogenous ligands of Liver X Receptor α and β isoforms (LXRs). Given the action of LXRs as whole-body cholesterol sensors and key regulators of lipogenesis, oxysterols have the potential to assume a key role in the modulation of diverse pathways in lipid metabolism, glucose homeostasis, reproduction, development, inflammation, and immunity. Accordingly, LXRs and their ligands are being intensely studied as potential therapeutic targets for diverse diseases such as lipid disorders, chronic inflammation, autoimmunity, neurodegenerative disease, and cancer. Up to now, development of LXR modulators has been mainly focused on non-steroidal compounds. Although some non-steroidal, high potency compounds have been discovered (e.g., T0901317, GW3965), they demonstrate a low or null gene-selectivity and induce lipogenic effects by increasing liver and circulating triglyceride levels. Consequently, these compounds have limited clinical use, thus creating an opportunity in the market for steroid based LXR modulators endowed with higher specificity and less toxicity.

PRODUCT & TECHNOLOGY

The present invention relates to a portfolio of 16 novel steroidal stigmasterol and ergosterol derivatives that function as potential Liver X Receptor (LXR) agonists for the treatment of diseases associated with LXR, such as cancer, inflammation, metabolic and autoimmune diseases. LXR agonists induce the expression of target genes (i.e. ABCA1), which are involved in cholesterol homeostasis. In the liver, LXR activation promotes the biosynthesis of fatty acids by inducing the expression of SREBP-1c, as well as several downstream genes in the SREBP-1c pathway, including FASN and SCD1. Importantly, no compound up-regulated the mRNA levels of FASN and SCD1.

SOLUTION & BENEFITS

The novel steroidal stigmasterol and ergosterol derivatives here described could be employed as potential Liver X Receptor (LXR) agonist for the treatment of diseases associated with LXR, such as cancer, inflammation, metabolic, and autoimmune diseases. In particular, these compounds show (i) high LXR selectivity, (ii) high gene selectivity for cholesterol homeostasis rather than for lipogenesis, and (iii) less toxic effect than non-steroidal compounds. Indeed, substantial efforts have been dedicated to the identification of LXR ligands able to turn on ABC transporter genes, without affecting lipogenic genes levels. In this context, the inventors demonstrated that all sixteen different compounds produced and tested were able to induce ABCA1 expression, with a particularly strong induction by four specific compounds when compared to a positive control (T0901317). According to pre-clinical experimental evidence, the derivatives 13, 19, 20, and 25, being strong inducers of ABCA1 and poor activators of SREBP1c and SCD1 in the U937 cell line, proved to be very promising derivatives for further clinical development.

MARKET & COMPETITION

We are currently seeking potential commercial partners with a strong pipeline in LXR modulators and small molecule therapeutics to develop new therapeutic agents for the treatment of LXR-related diseases. Currently, Lead Pharma, Innovimmune, and Inspirna are developing LXR agonists for diverse applications; from dermatology to cancer, with the furthest in a Phase II clinical trial for SCLC (NCT02922764).

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Side-Chain Modified Ergosterol and Stigmasterol Derivatives as Liver X Receptor modulators	WO2019/021122 US20200157135	Pending	EP USA

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Sector
Cancer

Key Publications
Raccosta et al. Cell Death & Dis. 2023.
[1]Tavazoie et al. Cell. 2018.

Development stage
Preclinical Development

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

Liver X Receptor (LXR) antagonists able to induce anti-tumor immune response

CLINICAL NEED

The outstanding success of immune checkpoint inhibitors (ICIs) in cancer treatment has clearly revealed the pivotal role of patient immune cells in tumor rejection and definitely established that anti-tumor therapy efficacy relies on its ability to trigger immune response. Although the success of ICI-based cancer immunotherapy, still many patients do not respond or progress after an initial benefit. Therapy-resistant patients often display signatures of poor T cell tumor infiltration and activation. Indeed, tumor-infiltrating T cells are key players in tumor debulking as they can recognize tumor-specific antigens and release cytotoxic cytokines. It is well known that effective T cell activation strongly depends on tumor-derived antigen presentation by professional antigen presenting cells such as dendritic cells (DCs). Immunosuppressive actions exerts by tumor cells affect DC differentiation and antigen presentation, dampening anti-tumor immunity. Many tumor products from cholesterol and lipid metabolism are responsible of this suppression. For this reason, a strategy to perturb intra-tumor lipid pathway and favor DC functionality would be potentially exploitable for cancer patient treatment.

SOLUTION & BENEFITS

Dr. Vincenzo Russo has developed two new synthetic antagonists of Liver X Receptors (LXRs), named PFM037 and PFM046, that are able to induce lipidome reprogramming, reduce levels of cellular cholesterol/cholesterol derivatives and glycerophospholipids, and increase levels of polyunsaturated fatty acid precursors of lipid mediators endowed with pro-inflammatory activity. By interfering with LXR signaling, PFM037 and PFM046 are able to:

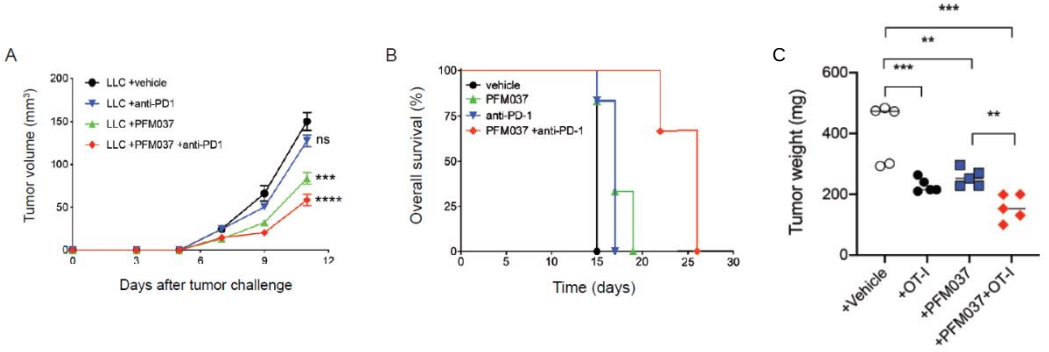
- Promote intratumoral monocyte-DC differentiation
- Increase number of tumor-infiltrating DCs
- Increase number of tumor-infiltrating cytotoxic T cells
- Delay tumor growth
- Synergize with anti-PD-1 and adoptive T cell therapy

Of note, these compounds might be also used in combination with our proprietary LXR agonists, reported in the WO2019/021122 patent, particularly those activating LXRβ isoform, which are endowed with the capacity to decrease the intratumor number of myeloid-derived suppressor cells[1].

Moreover, inventors also found that PFM037 induced phenotypic changes of activated T cell expanded *in vitro* with IL-7/IL-15. In particular, T cells cultured in presence of PFM037 and IL-7/IL-15 showed higher levels of markers associated to stem cell memory T cell endowed with a stronger antitumor potential.

PRECLINICAL RESULTS

Tumor-bearing mice treated with PFM037 and anti-PD-1 mAbs control tumor growth and had higher overall survival, suggesting that PFM037 could be used alone or in combination with immune checkpoint blockers (ICBs) (e.g. anti-PD-1 mAb) for cancer treatment (Fig. A-B). Moreover, the inventors observed a better control of tumor growth in mice bearing the melanoma and treated with the combination of PFM037 and in adoptive T cells (Fig. C). Thus, indicating that PFM037 treatments reprogram the hostile tumor microenvironment into an immune sensitive one.



INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
LXR antagonists	Not yet published	Pending	EU

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Sector
Cancer

Key Publications
Nardelli et al. Chem Comm 2019;

Development stage
Preclinical Development

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

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Gruppo San Donato

Peptide-based diagnostic tool for avb6 avb8-positive cancer imaging

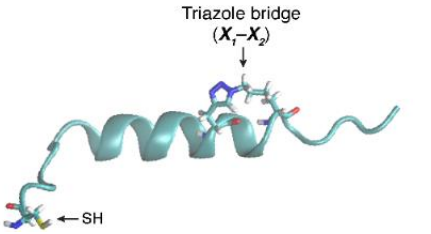
DIAGNOSTIC NEED

Integrins αvβ6 and αvβ8 are upregulated in many cancers, including pancreatic adenocarcinomas, oral mucosal and bladder cancers and melanomas, thus representing potential targets for diagnostic/theranostic purposes.

PRODUCT & TECHNOLOGY

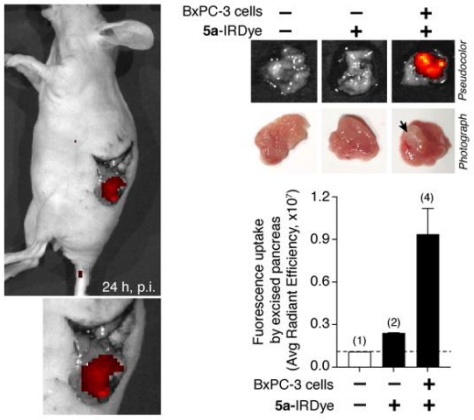
We have developed a 25-mer peptide, derived from the 39-63 region of human chromogranin A, which contains an RGDL motif followed by an alpha-helix chemically stapled with a triazole bridge. Peptide 5a represents the strongest bi-selective ligand for αvβ6/αvβ8 described to date (K_d: αvβ6: 0.63 ± 0.13 nM, K_d αvβ8: 3.19 ± 1.20 nM). NMR and computational/biochemical studies showed that peptide 5a binds the RGD binding site of αvβ6/αvβ8 with receptor–ligand interactions similar to those observed for the pro-TGFβ1/αvβ6 complex. Peptide 5a can be efficiently coupled via maleimide chemistry to the thiol group of the unique N-terminal cysteine to various compounds, including imaging tracers, nanoparticles, and proteins, without losing affinity for its targets.

Peptide **5a**: CFETLRGDLRILSILRX_1QNLX_2KELQD$_{CONH_2}$



PRECLINICAL RESULTS

Peptide 5a was labeled with optical- and radio-imaging compounds currently used in the clinical setting, such as IRDye® 800CW, a near-infrared (NIR) fluorescent dye, and with ^{18}F-NOTA, a tracer for positron emission tomography (PET). *In vivo* dynamic and static optical NIR and PET/CT imaging studies, performed in mice with subcutaneous and orthotopic αvβ6-positive carcinomas of the pancreas, showed high target-specific uptake of the fluorescence- and radio-labeled peptide by tumors (Fig. 1 A and B, below). Significant target-specific uptake of the fluorescence-labeled peptide was also observed in mice bearing subcutaneous αvβ8-positive prostate tumors (unpublished preliminary data). These results indicate that peptide 5a specifically targets to αvβ6- and/or αvβ8-positive tumors and can be exploited for imaging αvβ6 and/or αvβ8 positive tumors.



*Representative fluorescence image of the exposed pancreas of mice bearing BxPC3 orthotopic pancreatic adenocarcinoma injected with **5a**-IRDye.*

COMPETITIVE ADVANTAGES

- Human-derived peptide, lower risk of immunogenicity compared to peptides containing viral sequences.
- Binding affinity for αvβ6 and αvβ8 in the range of sub-low nM.
- Suitable for surgeon platforms in the context of image-guided surgery.
- Versatile ligand for diagnostic purposes: fluorescent dyes and radiotracers.
- Delivery of imaging or therapeutic agents to αvβ6/αvβ8 single- or double-positive tumors.

MARKET & COMPETITION

The global surgical imaging market is projected to reach USD 2.4 billion by 2025 from an estimated USD 1.8 billion in 2020, at a CAGR of 6.3% during the forecast period*. The major factors driving the growth of this market include the technological advancements, reimbursement cuts for analog radiography systems, and the increasing demand for minimally invasive procedures

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Chromogranin A-derived peptides and uses thereof	WO2021094608	Pending	PCT contracting states

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Sector
Cancer

Key Publications
Calcinotto, Brevi et al. Nat. Commun. 2018.
Bellone et al. Microbiol. Mol. Biol. Rev. 2020.
Brevi et al. Front. Immunol. 2020.

Development stage
Preclinical Development

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

Prevotella melaninogenica for the treatment of multiple myeloma

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

CLINICAL NEED

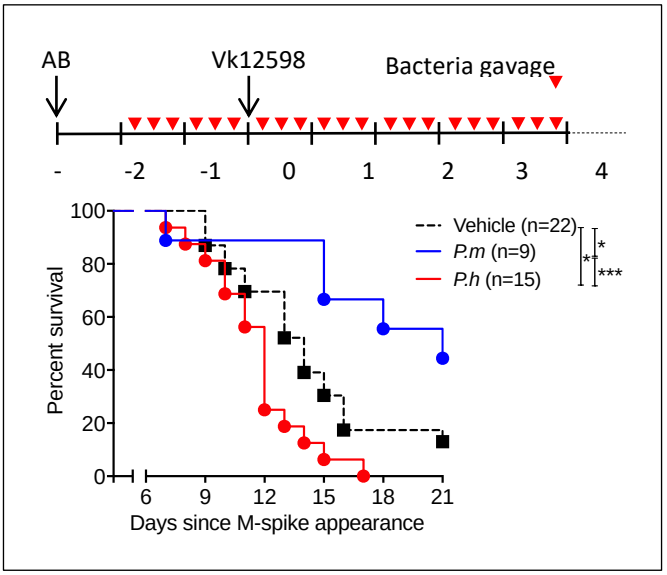
Multiple myeloma (MM) is a treatable, yet incurable plasma cell neoplasia. MM is often preceded by monoclonal gammopathy of undetermined significance (MGUS) or by smoldering multiple myeloma (SMM). MGUS and SMM are asymptomatic but diagnosable form.
Our treatment is particularly effective for SMM: patients are only monitored and no treatment given pending disease progression to symptomatic Multiple Myeloma, with a probability of 10%/year (unmet clinical need).

PRODUCT & TECHNOLOGY

Prevotella melaninogenica is a species of human commensal bacteria that can be administered orally as gut microbiota modulators (i.e. probiotic). *P. melaninogenica* has a proven utility as an agent capable of redirecting the local and systemic immune response toward a non-inflammatory type.

PRECLINICAL RESULTS

In a primary mouse model of MM, we showed that modulation of the gut microbiota by oral administration of the human commmensal *P. melaninogenica* delayed disease progression and associated with a reduced representation of Th17 lymphocytes both in the gut and in the bone marrow. Conversely, treatment with *P. heparinolytica* induced expansion of gut-born Th17 cells that migrated to the bone marrow and eventually propelled MM progression. Mechanistically, dendritic cells enriched from the bone marrow of mice treated with *P. heparinolytca* produced more pro-Th17 cytokines than dendritic cells from mice gavaged with *P. melaninogenica*. Similar results were obtained when human monocyte-derived dendritc cells were challenged with the *Prevotella* strains. The picture shows treatment schedule and overall survival (Kaplan-Meier plot) of t-Vk*MYC MM gavaged with vehicle (Vehicle), *P. heparinolytica* (P.h) or *P melaninogenica* (P.m). (Calcinotto, Brevi et al. Nat Commun 2018). In ongoing experiments, treatment with *P. melaninogenica* increased the therapeutic index of anti-PD-L1 antibodies and prevented appearance of symptomatic MM.



MARKET & COMPETITION

The MM market is expected to grow from $14.5bn in 2017 to $27.8bn by 2027 across the eight major markets at a compound annual growth rate (CAGR) of 6.7%*.
Our suggested first indication is for SMM but the treatment can also be extended to patients with MGUS that affects 3-4% of the population over the age of 50.
Regarding competition, MGUS and SMM patients aren't currently subject to any treatment since they are under an observational state. Considering IL-17 as key player involved in the progression of MM from MGUS/SMM, it is currently under development CJM-112 (Novartis AG) which acts by inhibiting interleukin-17, a cytokine that controls cells and activates inflammation. IL-17 is a key product of Th17 cells.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Bacterial strains for medical use	WO2020109620 EP3886881	Pending	EP US

(*) GlobalData(2019),EpiCastReport: Multiple Myeloma Epidemiology

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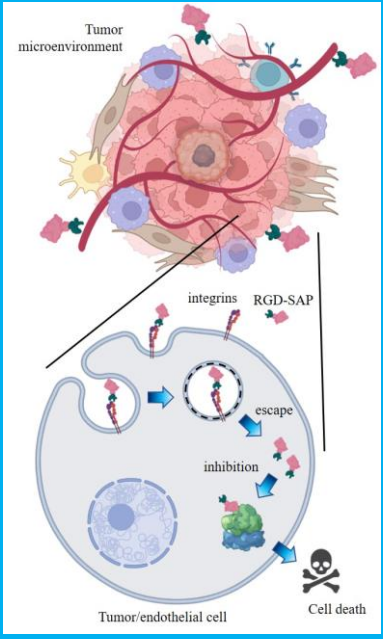
Key Publications
S. Zuppone et al. Frontiers in Oncology, 2022.

Development stage
Preclinical Development

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

The Technology



RGD-Saporin: a novel therapeutic agent based on a targeted-delivered toxin to inhibit tumor growth

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

CLINICAL NEED

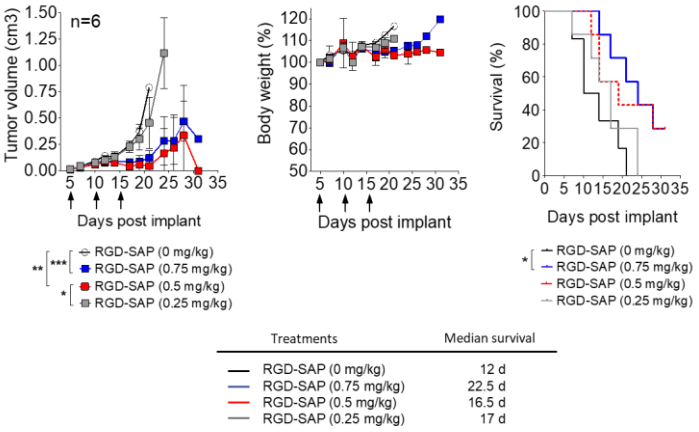
Current clinical protocols for the treatment of tumors are mostly based on surgical debulking, followed by radiation and chemotherapy. These types of therapies suffer from a lack of specificity, killing cells in a cell cycle dependent manner and causing an increased toxicity. Therapies based on toxins have gained great attention in the last decades, as they opened up the possibility to specifically deliver drugs to, and kill, cancer cells without, or minimally, affecting healthy organs.

PRODUCT & TECHNOLOGY

This technology is based on a chimeric recombinant protein formed by a tumor-targeting RGD sequence that enables specific and selective for αv-integrins, and a ribosome inactivating protein saporin (SAP) with a potent and efficient cytotoxic effect and an unusual resistance to high temperature, denaturation and proteolysis. The resulting protein (called RGD-SAP) can kill cells expressing αv-integrins. The αvb3, αvb5 and αvb6 significantly over-expressed in tumor context in a stage and grade-dependent manner lend support to the hypothesis that this class of receptors may represent important molecular targets for toxin delivery to cancer. RGD-SAP can be easily produced in E.coli.

PRECLINICAL RESULTS

The results of in vivo studies of bladder cancer in two different mouse models show that RGD-SAP can reduce tumor growth and significantly prolong animal survival without inducing detectable side effects. The inventors used a subcutaneous cancer model to determine the optimal dosage and found that RGD-SAP can inhibit tumor growth in a dose-dependent manner.



Treatments	Median survival
RGD-SAP (0 mg/kg)	12 d
RGD-SAP (0.75 mg/kg)	22.5 d
RGD-SAP (0.5 mg/kg)	16.5 d
RGD-SAP (0.25 mg/kg)	17 d

Due to its potential effects on tumor cells and microenvironment, RGD-SAP may represent a good therapeutic tool for cancer. In addition, by inhibiting protein synthesis, SAP acts in a cell cycle independent manner, thus targeting both quiescent and rapidly dividing tumor cells. This feature makes it suitable to contrast both aggressively growing cancers and tumors with slower progression. RGD-SAP can also be employed in combination with other therapeutic options based on different mechanisms of action, e.g. inhibition of DNA synthesis, cell division, and signal transduction. Dose dependent effects of RGD-SAP on tumor growth in a subcutaneous syngeneic bladder cancer mouse model are shown in the following figures.

COMPETIVIVE ADVANTAGE

- RGD-SAP high intrinsic activity
- Effects on tumor cells and microenvironment
- Cell cycle independent activity and effective on a universally conserved loop of rRNA
- Easy and rapid production (prokaryotic hosts, no renaturation after extraction)
- Active alone or in combination other therapeutic agents

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Fusion proteins and uses thereof	Not yet published	Pending	Italy

Biomaterial Coated Gold Nanostructures for Photoacoustic Imaging and Photothermal Therapy of Tumor Lesions

I.R.C.C.S. Ospedale San Raffaele
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Sector
Oncology
Urology

Key Publications
Alchera E. Photoacoustics 2022

Development stage
Preclinical

Business Model
Out-licensing for further development and subsequent commercialization.

CLINICAL NEED

Several imaging methods have been used for the diagnosis of Bladder Cancer (BCa). Being a hollow organ surrounded by other organs, the bladder continuously changes its volume and has an irregular shape. As a consequence of these limitations the detection rate of BCa by US and CT scan vary by the bladder region and is very poor for BCa <5 mm size. Cystoscopy remains the gold standard diagnostic method for patients with BCa symptoms or during surveillance, and cannot be replaced by cytology or by any other non-invasive test. For lesions <1 mm of size, a diagnosis of Cis cannot be made with imaging methods alone, and even cystoscopy, including photodynamic diagnosis, has limited diagnostic utility for Cis. As a consequence of these limitations preventing the recognition of small lesions, residual high grade disease is found in 40% of patients after the first transurethral resection of bladder tumor (TURBT); patients with BCa experience a very high frequency of relapse in the years following the first diagnosis, thus undergoing follow-ups for the rest of life and not very effective treatments, with a consequent poor quality of life and the highest cost per patient among all cancers.

PRODUCT & TECHNOLOGY

To overcome the limitations of the imaging methods currently used in the clinics for BCa, our researchers developed a novel approach for a non-invasive early diagnosis of in vivo orthotropic bladder cancer, by exploiting an imaging modality based on the photoacoustic (PA) imaging (PAI) approach.
The new technological platform is based on:

1. Gold Nanoparticles that have been chemically engineered with chitosan and the peptide Iso4, to enable tumor targeting;
2. The intravesical instillation of urine-stable targeted GNRs
3. A technique of US-assisted shaking of GNRs@Chit-Iso4, to prevent nanoparticle sedimentation in the bladder;
4. The multimodal imaging of cancer lesions with PAI.

The current approach can be used both to detect the tumor, and to treat it, through GNR mediated thermal ablation.

SOLUTION & BENEFITS

This novel strategy, that combines multiple technologies, allows for the detection of BCa with unprecedented sensibility. Indeed, with this platform makes it possible to detect neoplastic lesions smaller than half a millimeter, with a sensitivity that far exceeds that of US and CT urography for bladder carcinoma.
Detection and removal of bladder lesions <1mm will improve the quality of life of millions of people while reducing social costs.

MARKET

Bladder cancer is the 10th most common cancer type worldwide. Every year, about 600 000 people are diagnosed with bladder cancer worldwide and more than 200 000 people die from this disease. Bladder cancer is one of the most challenging and expensive cancers to diagnose and treat. Most bladder cancers are diagnosed at an early stage, when they are highly treatable. However, about 25% of bladder cancers are diagnosed at later stages.
The size of the global bladder cancer therapeutics market is expected to grow to USD 336.8 million by 2023, from USD 264.5 million in 2023.

INTELLECTUAL PROPERTY

Priority patent application (undisclosed number).

Cell therapy

OSPEDALE
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Sector
Cell therapy

Key Publication
Comi et al. Front. Immunol. 2020.

Development stage
In vivo proof-of-concept

Business Model
Out-licensing for further development and subsequent commercialization.

The Technology
Engineered Dendritic Cells, generated from a donor of transplanted organ, over-expressing IL-10.

Dendritic cell-based immunotherapy to prevent graft rejection after transplantation

CLINICAL NEED

80.926 kidney and 32.586 liver transplants have been performed worldwide in 2020; overall, acute graft rejection occurs in ~15-20% of kidney and 20-40% of liver transplants. The drastic expansion in the immunosuppressive drugs repertoire led to a better control of acute rejection. Unfortunately, the side effects of these drugs can be severe, which is one of the reasons why life expectancy of transplant patients still significantly falls short of that of the general population. Moreover, these drugs, and calcineurin inhibitor-based regimens, are ineffective in preventing late allograft loss. Therefore, the improvement of the survival of both the graft and the patient is a strong medical need.
Current treatments to prevent rejection after allogenic organ transplantation are:

- Long-term immunosuppressive regimens – 8 approved drugs
- Innovative therapies based on regulatory cell immunotherapy:
  - T regulatory cell (Treg)-based therapies
  - Myeloid regulatory cell (MRC)-based therapies.

PRODUCT & TECHNOLOGY

The approach consists in Lentiviral Vector-mediated transfer of IL-10 in combination with a marker gene to generate stable engineered donor-derived dendritic cells suitable for cell-based approaches to prevent organ rejection after transplantation.

SOLUTION & BENEFITS

LV-mediated gene transfer of IL-10 in DC (DC^{IL-10}) has the potential to overcome the major limitations of Treg-based therapies and to be more effective compared to other MRCs, as it will result in a drug product that will:

- Induce allo-specific immunological non-responsiveness in effector T cells;
- Promote a self-reinforcing peripheral regulation, with the induction of allo-specific Tregs *in vivo* in a physiological manner;
- Have a limited life span *in vivo* (up to 14 days), overall limiting the long-lasting impact on immunity against infections and malignancies;
- Promote stable over-expression of IL-10 ensuring the generation of a local microenvironment enriched in IL-10, which modulates T cells, myeloid cells, and innate cells, sustaining long-term tolerance.

MARKET & COMPETITION

Despite being effective, *standard immunosuppressive regimens* require long-term treatments, which are associated with several side effects, and the current life expectancy of kidney transplanted patients is still significantly short compared to that of the general population. Immunosuppressive treatments are administered every day leading to an annual cost 14K$.

With respect to *Innovative therapies based on regulatory cell therapy*, instead, ongoing clinical trials with Treg-based therapy demonstrated the safety of the approach and some clinical benefit. However, several open issues remain to be solved, such as: i) the potential of polyclonal *in vitro* expanded Tregs to mediate pan immunosuppression *in vivo*. For this reason, pre-clinical studies are ongoing to generate antigen-specific Tregs to limit this side effect. ii) Infused Tregs are subject to potential destabilization in strong inflammatory conditions *in vivo* and adopt pathogenic effector T phenotype and functions, thereby possibly mediating graft rejection. iii) The overall impact of long-lasting Tregs on hampering immunity against infections and malignancies. Only few patients have been treated with MRCs (i.e., Mregs or TolDC). Thus far, published data on a small number of transplanted patients demonstrated the safety of the approach and showed that infusion of donor-derived Mregs in kidney-transplanted patients allows tapering of immunosuppressive regimen and induction of Tregs *in vivo*.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Production of engineered cells and uses thereof	WO2019243461	Pending	Europe Canada China Japan Australia India

Use of antigen-specific tolerogenic-dendritic cell for the treatment of autoimmune disease

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Sector
Cell therapy

Key Publication
Manuscript under revision

Development stage
In vivo proof-of-concept in two disease models, i.e., type-1 diabetes and celiac disease.

Business Model
Out-licensing for further development and subsequent commercialization.

The Technology
Engineered Dendritic Cells, generated from patient affected by an autoimmune disease, that stably present the antigen of interest in the presence of sustained levels of IL-10 to promote/restore long-term tolerance via antigen-specific T regulatory cells.

CLINICAL NEED

Failure of one or more players involved in the mechanisms sustaining immune tolerance to self or non-harmful antigens (Ags) can cause unwanted immune responses, leading to the development of T cell mediated diseases, such as autoimmunity. It is estimated that 1 in 10 individuals suffers from autoimmune diseases worldwide. Currently approved therapies for the management of these patients vary from administration of immunosuppressive drugs to supportive therapies. Although current therapies control symptoms of the disease, they negatively affect the patients' quality of life and are not devoid of side effects. For example, general immune suppression leads to increased risk of infections or cancer development. In addition, these therapies are given lifelong to partially compensate for organ dysfunction, but they do not treat the cause of the disease and therefore they are not curative.

PRODUCT & TECHNOLOGY

The technology consists in dendritic cells (DC) transduced with lentiviral vectors (LVs) encoding for both human (h)IL-10 and specific autoimmune disease-associated peptides fused to the invariant chain ($DC^{IL\text{-}10/Ag}$). These $DC^{IL\text{-}10/Ag}$ cells secrete supra-physiological levels of IL-10, down regulate pathogenic CD4+ and CD8+ T cell responses *in vitro* and induce Ag-specific type 1 T regulatory (Tr1) cells *in vivo*, preventing disease development in pre-clinical models of Type 1 Diabetes (T1D).

SOLUTION & BENEFITS

In vivo administration of $DC^{IL\text{-}10/Ag}$ not only controlled T effector cell responses *in vitro* and T effector cell mediated tissue damage *in vivo*, but also initiated an Ag-specific self-sustaining tolerogenic loop, likely mediated by long-term Ag-specific Tr1 cells. Overall, this technology represents a step forward in tolerogenic DC-based approaches since IL-10-engineered DC better target pathogenic responses and promote long-term tolerance due to their unique ability to induce Ag-specific Tr1 cells. Added to this, $DC^{IL\text{-}10/Ag}$ efficiently controlled CD8+ T effector functions against model Ags in mice. In a mouse model of type-1 diabetes, $DC^{IL\text{-}10/Insulin}$ could control T1D onset in the presence of diabetogenic CD8+ T cells, thus supporting the idea that persistent Ag-stimulation concomitant to IL-10 supplementation enhances CD8+ T cell dysfunction. Blockade of Ag-specific pathogenic CD8+ T effector cells represents a key advantage of the $DC^{IL\text{-}10/Ag}$ platform, not previously reported for DC-based cell therapy.

COMPETITIVE LANDSCAPE & ADVANTAGES

Innovative therapies based on *ex vivo* expanded polyclonal Tregs have been tested to cure autoimmune diseases, including Type 1 Diabetes. Treg-based therapy showed improved beta-cell function and reduced exogenous insulin requirement only in the short-term. The partial efficacy of Treg-based therapy may depend on the limited residual functional beta-cells at time of treatment, the inadequate *in vivo* availability of IL-2, the key cytokine for Treg survival and expansion, or, more importantly, on the lack of autoantigen-specificity of infused Tregs. Supporting the latter, reports in pre-clinical models indicated that antigen-specific Tregs are more effective than polyclonal Tregs in restoring tolerance in Type 1 Diabetes. Our antigen-specific, IL-10 secreting tolerogenic DCs have the potential to overcome the major limitations of the abovementioned technologies, as it will result in:

- stable expression and presentation of the antigen of interest to both CD4+ and CD8+ T cells, thus promoting antigen-specific immunological unresponsiveness;
- concurrent modulation of antigen-specific pathogenic T cells and induction of/conversion into antigen-specific Tregs *in vivo* in a physiological manner;
- stable preservation of tolerogenic properties *in vivo*, even in a pro-inflammatory environment;
- a limited life span *in vivo* (up to 14 days), overall limiting the long-lasting impact on immunity against infections and malignancies;
- local delivery of IL-10 that will inhibit both T cells and myeloid cells, and innate cells, thus sustaining tolerance.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Production of engineered cells and uses thereof	WO2019243461	Pending	Europe Canada China Japan Australia India

Skeletal muscle progenitors for the treatment of Duchenne Muscular Dystrophy

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Sector
Cell Therapy

Key Publication
Cossu et al., EMBO Mol. Med. 2015

Development stage
Phase I/II

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

CLINICAL NEED

Several genetic diseases affect mesoderm tissues such as skeletal and smooth muscle, cartilage, bone, joints, and the wall of blood vessels. Many of them are rare, not well studied, and are devastating conditions that dramatically affect numerous organs, and compromise life quality and expectancy. All lack an efficacious therapy.
Among genetic diseases affecting skeletal muscle, Duchenne Muscular Dystrophy (DMD) is the most common, affecting 1/4,000 newborn males. It is caused by mutations in the dystrophin gene, located on X chromosome, and characterized by progressive deterioration of skeletal and cardiac muscle. This quickly leads to a variable but progressive limitation of the patient's mobility, including confinement to a wheelchair and heart and/or respiratory failure typically by 30-40 years of age. Despite numerous attempts with oligonucleotides aimed at correcting the mutated mRNA and AAV delivering micro-dystrophins (shorter and partially functioning proteins), DMD is still incurable and does not yet have a therapy with long lasting/curative benefits

PRODUCT & TECHNOLOGY

The current invention aims at developing a range of treatments for DMD patients with various genotype backgrounds by combining cell therapy with exon skipping to exploit the advantages of both and compensate their respective drawbacks. We have compelling in vitro and in vivo evidence of dystrophin production above 30% of healthy muscle, considered a therapeutic level. While the commercial potential of cell therapies has always been limited due to the risk of immune rejection, the current invention overcomes this limitation by using engineered multipotent cells, called mesoangioblasts (immune privileged universal donor cells), which were developed as an allogenic product for DMD patients affected with the same genetic mutation.
With this approach, we aim to create GMP grade, off the shelf, immune privileged, universal donor mesoangioblasts, vessel associated myogenic progenitors. Thanks to a newly discovered method to indefinitely expand human mesoangioblasts without immortalizing agents, these will be genome-edited to delete HLA and create a bank of cells available to be engineered to treat many monogenic diseases affecting the mesoderm, thus cutting cost. As proof of principle, cells will be engineered to treat Duchenne and Congenital muscular dystrophies, and a similar approach has been envisioned for merosin-deficient congenital muscular dystrophy type-1A (MDC1A) and Limb Girdle Muscular Dystrophy 2C (LGMD2C) for which pre-clinical models have already been validated.

SOLUTION & BENEFITS

This is the first characterization of a human cell population that fulfils all the criteria of a successful cell therapy protocol in Duchenne Muscular Dystrophy and has demonstrated several clear advantages over the current standard of care:

- Efficient engraftment of treated cells in muscles
- Amplification of treatment along the multinucleated muscle fibres: each engrafted nucleus produces a small nuclear RNA that diffuses to and induces exon skipping also in neighboring nuclei
- Development of a single cell therapy product for the treatment of whole populations with the same genetic defect (unlike autologous approaches)
- Pre-clinical evidence of a long lasting effect
- Previous experience of phase I/IIa clinical trials and ongoing proof of concept trial.

The nature of the approach is flexible and modular in addressing gene disruption in many mesoderm genetic diseases. It may be extended to other muscular dystrophies and to rare diseases of the connective tissue that are due to mutations in genes encoding for proteins of the extra-cellular matrix.

MARKET & COMPETITION

Currently, of the 16 products marketed for muscular dystrophies, all are small molecules and none are classified as gene or cell therapy. Sarepta Therapeutics leads the way in DMD with several gene therapies being developed. The forecasted global sales is expected to grow from USD 117M in 2023 to USD 4.1B in 2028.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Skeletal muscle periangioblasts and cardiac mesoangioblasts, method for isolation and uses thereof	WO2007093412	Active	EP US

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Sector
Cell Therapy

Key Publications
Pluchino et al., Nature 2005;
Martino G, Pluchino S. Nat Rev Neurosci. 2006

Development stage
Phase I

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

Use of neural stem cells to induce neuro protection in inflammatory CNS disorders

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

CLINICAL NEED

Transplantation of neural stem precursor cells in patients affected by central nervous system (CNS) disorders characterized by chronic inflammation (e.g., multiple sclerosis, brain tumors, ischemic stroke) has limited therapeutic impact due to recurrent or persisting inflammation that targets and kills both CNS-resident and transplanted cells. Consequently, there is a strong clinical need for immunomodulatory therapies that avoid inflammatory self-reactivity and boost neuronal protection.

PRODUCT & TECHNOLOGY

We have developed a novel immunomodulatory mechanism that boosts such limited therapeutic effect by transplanting undifferentiated adult neural stem/progenitor cells (aNPC), which promote direct neural cells replacement by acquiring *in vivo* terminally differentiated phenotype. As a proof of concept, subventricular zone (SVZ)-derived syngenic adult NPCs (aNPC) were transplanted in a mouse model of chronic-recurrent autoimmune CNS inflammation, namely relapsing-remitting experimental autoimmune encephalomyelitis (R-EAE). While assessing their therapeutic potential, they demonstrated that during R-EAE inflamed CNS, perivascular areas function as ideal, although atypical, niche-like microenvironments where transplanted cells can survive for long-term (up to 3 months post-transplantation) as bona fide aNPCs. Furthermore, upon systemic injection, aNPC can exert a neuroprotective effect by inducing *in situ* programmed cell death of blood-borne CNS-infiltrating pro-inflammatory Th1, without affecting anti- inflammatory Th2 cells in the inflamed CNS perivascular area. Thus, the CNS inflammatory microenvironment dictates aNPCs cell fate, and therefore their therapeutic efficacy is as follows:

- when neurodegeneration prevails, transplanted aNPCs acquire a mature phenotype and thus replace damaged neural cells, while
- when neuroinflammation predominates, transplanted aNPCs survive to recurrent inflammatory episodes by retaining both an undifferentiated phenotype and notable proliferating capacities.

SOLUTION & BENEFITS

Undifferentiated aNPCs have relevant therapeutic potential in chronic inflammatory CNS disorders because they display immune-like functions that promote long-lasting neuroprotection in inflamed CNS perivascular area on the one hand, and brain repair on the other. Among their competitive advantages, they have:

- aNPC-mediated apoptosis of blood-borne CNS-infiltrating encephalitogenic T cells, promoting long-lasting neuroprotection in chronic inflammatory CNS disorders;
- Selective accumulation of intravenously-injected aNPCs within CNS inflamed areas using constitutively functional homing molecules (e.g., a4 integrins and GPCRs) canonically used by pathogenic CNS- infiltrating blood-borne lympho- and monocytes;
- Preferential maintenance of an undifferentiated phenotype upon aNPCs transplantation, thus potentially escaping the chronic CNS-reactive autoimmunity;
- In vivo maintenance of their proliferation capacity for up to 100 days after aNPCs transplantation, thus potentially modulating their fate in vivo (proliferation vs quiescence vs migration and differentiation) in response to specific environmental signals (e.g., cytokines, chemokines, stem cell regulators).

MARKET & COMPETITION

The global market for CNS disorders in general is quite large, with 2,393 past and present deals for a total of over USD 70B in sales for 2021, and a forecasted market over USD 170B (representing a 14% CAGR). Regarding the subset of Multiple Sclerosis, a narrower market of around 300 past and ongoing deals with sales of around USD 21B for 2021 and an estimated USD 28B in 2028 (representing around 4% CAGR). In both cases, the vast majority of marketed products are small molecules, making this strategy and attractive alternative to currently available therapies.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Inflammation	WO2007015173	Active	EP JP

Gene therapy

New gene therapy approach to induce antigen-specific immunological tolerance

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Sector
Gene therapy

Key Publications

1) Russo F, et al. Diabetes. 2021.
2) Akbarpour M, et al. Tregs.Sci Transl Med. 2015.
3) Annoni A, et al. EMBO Mol Med. 2013.
4) Cantore A, et al. Blood. 2012.
5) Mátrai J, et al. Hepatology. 2011.
6) Annoni A, et al. Blood. 2009.
7) Brown BD, et al. Nat Rev Genet. 2009.

Development stage
Preclinical Development

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

CLINICAL NEED

In vivo induction of antigen-specific T regulatory cells (Treg) has been a long-sought goal of experimental medicine. This technology based on hepatocyte-targeted Integrase-Deficient Lentiviral Vectors (IDLV) allows in vivo induction of antigen-specific Treg and active immune tolerance against self or foreign antigens.

SOLUTION and BENEFITS

This platform enables efficient liver gene transfer for a window of time and induces immune tolerance to the encoded antigen in a “hit and run” approach, without the need for long-term integration, thus providing enhanced safety as compared to Integrase-Competent Lentiviral Vectors (ICLV). Hepatocyte-targeted IDLV are promising new vectors for a broad range of applications, and primarily for:

- induction of antigen-specific Treg in “inverse vaccination” strategies to tolerize individuals to protein replacement therapies (such as in lysosomal storage disorders and hemophilias or other plasma protein deficiencies);
- prevention or reversion the development of autoimmune diseases (such as multiple sclerosis, diabetes) and allergic diseases;
- reversible hepatic gene transfer of therapeutic proteins of therapeutic proteins, such as interferon (IFN) and other cytokines, in chronic viral hepatitis or hepatic tumors; gene-based delivery may provide therapeutic concentrations of the factor at the disease site with limited systemic exposure and only for a defined window of time.

STAGE OF DEVELOPMENT

In vivo proof of principle has been achieved with different model antigens (GFP, ovalbumin) and a therapeutic protein (coagulation factor IX in hemophilia B mice). Moreover administration of a hepatocyte-targeted IDLV enabled expression of insulin in hepatocytes and arrests pancreatic β-cell destruction in pre-diabetic NOD mice by generating insulin-specific FoxP3+ Treg[1].

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Gene Vector for Inducing Transgene-Specific Immune Tolerance	WO2010055413	Granted	US, EU

PROPRIETARY ENABLING TECHNOLOGIES

Technology	Development Stage
microRNA regulated transgene expression[7]	Clinical

IL2-based gene therapy for HBV treatment

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Sector
Gene Therapy

Key Publications
Bénéchet et al. Nature 2019.

Development stage
Lead Optimization

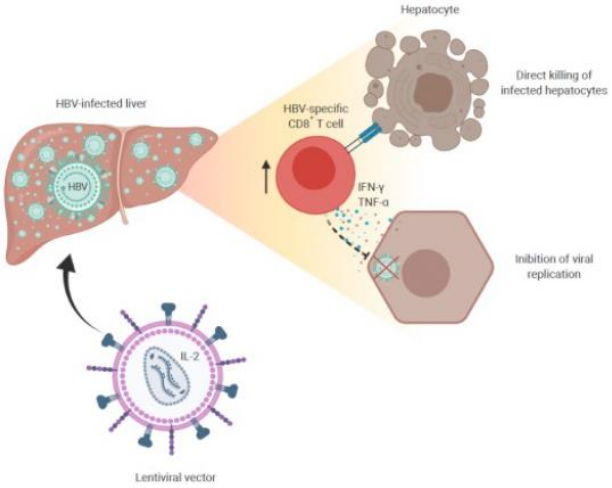
Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

CLINICAL NEED

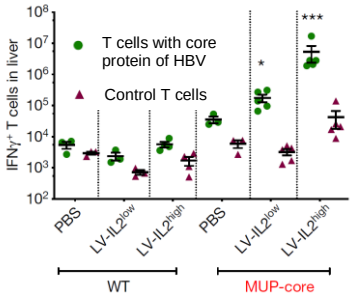
Hepatitis B virus (HBV) infection remain a major public health issue worldwide. Current treatment for HBV mainly relies on direct acting antiviral drugs, which suppress virus production, but do not eradicate HBV from the liver. The World Health Organization estimates that in 2015, 257 million people were chronically infected with the HBV virus globally (World Health Organization, 2020). Accordingly, this leads to a requirement for lifelong treatment.
In general, CD8+ T cells have a critical role in eliminating intracellular pathogens. The liver, for its specific features, is thought to be biased towards inducing a state of T cell unresponsiveness or dysfunction. This phenomenon underpins the unresponsiveness toward antigens specifically expressed in hepatocytes, and the propensity of some hepatotropic viruses, such as HBV, to establish persistent infections.

PRODUCT & TECHNOLOGY

We have found that administration of interleukin-2 (IL-2) enables reinvigoration and restoration of effector responses in dysfunctional CD8+ T cells, such as against antigens specifically expressed in hepatocytes. Moreover, the studies have revealed that local administration of IL-2 to the liver is able to increase the effector responses while avoiding the toxicity that is associated with systemic administration of IL-2.



PRECLINICAL RESULTS



To test the clinical potential of IL-2 in a system that would limit its systemic toxicity, we generated proprietary third-generation, self-inactivating lentiviral vectors (LVs) that selective hepatocellular expression of IL-2. We observed that the targeted delivery of IL-2 to the liver promotes the differentiation of HBV-specific dysfunctional CD8+ T cells into effector, IFNγ-producing cells endowed with antiviral potential. (Figure, left).

MARKET & COMPETITION

Chronic Hepatitis B is still considered a great global health burden even though there is an effective vaccine. A definitive treatment for HBV eradication is not yet available on the market. Conventional treatments are based on antiretroviral such as tenofovir, lamivudine, adefovir, entecavir or telbivudine. In this regard, the prevalent view in the scientific community is that, similarly to HIV infection, combination therapy (a “cocktail” of drugs) will be required to cure chronic HBV infection. The global chronic hepatitis B therapeutics market is expected to reach a value of $3 billion by 2024.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Agents and methods for treating viral infections	WO2020239964	Pending	EP US

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Sector
Gene Therapy

Development stage
Preclinical

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

The Technology
AAV vector with enhanced brain penetration and transduction

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

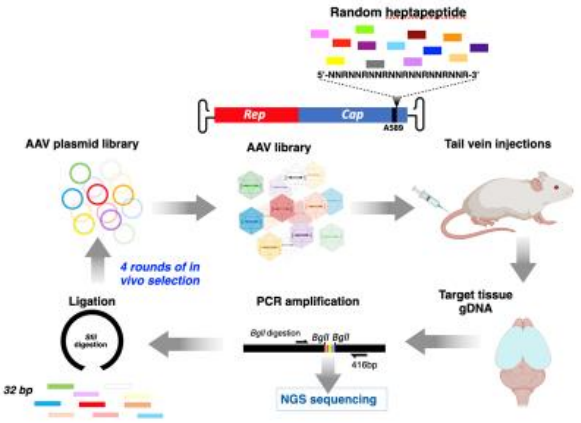
New Adeno-Associated Virus 9 (AAV9) capsid variants with enhanced brain targeting

CLINICAL NEED

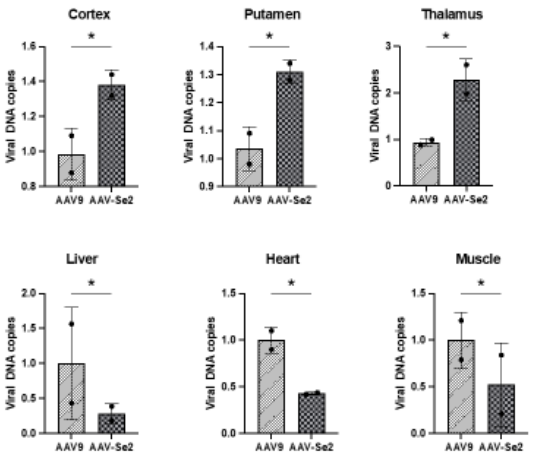
In order to achieve optimal delivery of the target tissue and avoid possible detrimental effects, gene therapy approaches require vectors endowed with both high transduction efficiency and specificity. Moreover, systemic delivery of the gene therapy formulation is preferable and more effective, but diffusion into the central nervous system (CNS) is limited by the blood-brain barrier (BBB) that restricts the passage of molecules, including AAV capsids. Since cerebral endothelial cells play a key role in BBB integrity and function, a recombinant AAV specifically targeting these cells could be helpful for the development of new therapeutic approaches for various CNS diseases. Accordingly, there is a significant need for vectors that not only target brain endothelial cells, but also cross them, thus reaching the cerebral parenchyma and infecting neurons and glia cells. The clinical relevance of such a tool would be invaluable and could bear innumerable applications in CNS pathologies.

PRODUCT & TECHNOLOGY

Starting from these premises, our researchers executed an in vivo screening approach using an AAV9 display peptide library to select novel brain targeting capsid variants. The peptide library was inserted in the AAV9 capsid with additional W503A mutation which is known to erase the AAV9 natural biding on galactose thus facilitating new interactions. In order to avoid Ly6a binding the selection was carried out using BALB/c mice that present mutations in the Ly6a locus that significantly decrement its expression. Three enriched capsid variants were identified after four consecutive rounds of selection. All of them displayed prevalent endothelial cell transduction that we identified and characterized on transduced mouse brains after tail vein injections. Brain cell transduction and neuronal targeting were also observed with interesting differences between brain regions with two capsid variants and equally conserved in BALB/c mice.



SOLUTION & BENEFITS



These targeted modifications in AAV9 capsids result in enhanced brain targeting and transduction capability. In particular, our researchers generated AAV9 capsid libraries and tested them for in vivo screening for new variants with enhanced transduction ability through consecutive rounds of selection for the desired tissue. They have discovered modifications of capsid proteins that enhance targeting to cells of the brain or central nervous system (e.g. increase the ability to cross the BBB), increase the transduction efficiency of these cells, and decrease tropism for non-brain tissue, when compared to AAV9. Moreover, the resulting capsid proteins have been validated in both mice and marmosets.

WHAT WE ARE LOOKING FOR

Industrial partners with experience in AAV technology interested in out-licensing this technology for commercialization and potentially further development with option rights.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Gene Therapy	Not yet available	Pending	Priority

Cell & gene therapy program

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

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Sector
Cell & gene therapy

Key Publications
1) Russo F, et al. Diabetes. 2021.
2) Akbarpour M, et al. Tregs.Sci Transl Med. 2015.
3) Annoni A, et al. EMBO Mol Med. 2013.
4) Cantore A, et al. Blood. 2012.
5) Mátrai J, et al. Hepatology.2011.
6) Annoni A, et al. Blood. 2009.
7) Passeri L, et al. J Autoim. 2023
8) Comi, et al.Front Immunol.2020.
9) under revision
10) Bénéchet, et al. Nature. 2019.
12) Piras F, et al. EMBO Mol Med. 2017
13) Petrillo C, et al. Cell Stem Cell. 2018
14) Milani M, et al. EMBO Mol Med. 2017
15) Zonari E, et al. Stem Cell Reports. 2017
16) Milani M, et al. Sci Transl Med. 2019
17) Brown BD, et al. Nat Rev Genet. 2009.

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

INTRODUCTION

Cell & Gene Therapy are innovative medical approaches that aim at treating various diseases by targeting the underlying genetic or cellular causes and thereby modify or replace dysfunctional cells or genes in the body.

Cell therapy involves using living cells to restore or improve the function of damaged tissues or organs; the cells can me modified, for example through genetic engineering, to enhance their therapeutic potential.

Gene therapy revolves around introducing genetic material into the patient's cells to correct or replace defective genes that are the culprits of specific conditions. The genetic material can be delivered through either viral or non-viral vectors, each with their pros and cons. There is still a lot of room for improvement and challenges remain, such as optimizing the delivery, ensuring the safety of the patients and managing the immune response.

CLINICAL ADVANTAGES

1. Targeted approach: the treatment can be tailored to the individual patient's needs; this allows for more precise and effective treatment.
2. Potential for permanent cure: they have the potential to provide a permanent cure to genetic disorders by correction the underlying mutation responsible for the disease.
3. Reduced need for repetitive treatments: especially relevant for cancer patients currently undergoing chemotherapy, cell & gene therapy may require only a few rounds over the patient's lifetime.
4. Personalized medicine: they can be customized to the specific patient's genetic makeup or disease features, which can improve treatment outcome and reduce the risk of adverse reactions.

CELL & GENE THERAPY AT SAN RAFFAELE UNIVERSITY HOSPITAL

San Raffaele University Hospital has always been at the forefront of cell & gene therapy for almost 30 years and has become one of the most advanced and productive gene therapy centers in the world. The Hospital has collaborated and initiated numerous clinical trials for a wide range of diseases, including genetic disorders, immune disorders, and cancer.
One of the most notable achievement is the development of a gene therapy treatment for ADA-SCID, which was approved with the name Strimvelis by the EMA in 2016.

AVAILABLE TECHNOLOGIES

At San Raffaele University Hospital, cell & gene therapy research is applied to multiple therapeutic areas and combining different approaches. This leads to the invention of different IPs that have translational potential in genetic diseases, cancer, neurological disorders, immune disorders, infectious diseases and metabolic disorders.

Category	Technology	Stage
Autoimmune diseases	Integrase-deficient LV inducing immunological tolerance[1-6]	Preclinical
	Antigen-specific tolerogenic dendritic cells for the treatment of autoimmune diseases[7] and graft rejection[8]	Preclinical
Liver cancer	LV-mediated IFNa delivery for liver metastases eradication[9]	Preclinical
Infectious diseases	IL2-based gene therapy for HBV treatment[10]	Lead opt.
Solid tumors and leukemia	CAR and TCR engineered T cells	Preclinical
Methods	Agents to improve efficiency of cell transduction and gene editing[12]	Clinical
	Agents to improve survival and engraftment of gene modified HSC[13]	Clinical
	Immune-shielded viral vectors[14]	Clinical
	Improved method for the genetic modification of HSC[15]	Preclinical
	Improved method for the transduction of antigen presenting cells[16]	Preclinical
	MicroRNA regulated transgene expression[17]	Clinical

Genetic disorders

Protein-based approach for Duchenne Muscular Dystrophy

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Sector
Genetic disorders

Key Publication
Careccia et al. Sci Transl Med. 2021.

Development stage
Preclinical

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

CLINICAL NEED

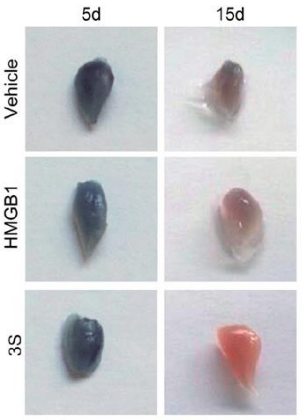
Duchenne Muscular Dystrophy (DMD) symptom onset is in early childhood, usually between ages 2 and 3. The disease primarily affects boys, but in rare cases it can affect girls. In Europe and North America, the prevalence is 6 per 100,000 individuals, incidence 1 per 3500/5000 live male birth. Limb-Girdle Muscular Dystrophy (LGMD) affects males and females in equal numbers; its prevalence is unknown, but estimates range from one in 14,500 to one in 123,000. The age of onset can vary greatly even among individuals of the same family. In all cases, muscular dystrophy is characterized by progressive muscle wasting associated to chronic local inflammation and oxidative stress. High Mobility Group Box 1 (HMGB1) is a nuclear protein that signals tissue damage when released into the extracellular medium We demonstrated that the oxidation of HMGB1 cysteines switches its extracellular activities from the orchestration of tissue regeneration to the exacerbation of inflammation. Specifically, oxidized HMGB1 acts as a proinflammatory mediator by interacting with Toll-Like Receptor 4 (TLR4) and the Receptor for Advanced Glycation Endproducts (RAGE) while reduced HMGB1 supports tissue regeneration through CXCR4 by acting on both the stem cells and their microenvironment. Currently, there is no cure for DMD and a very limited efficacy of current treatments (Steroids, ASO for Exon skipping, small molecule for read-through premature nonsense stop codon).

PRODUCT & TECHNOLOGY

We designed a non-oxidizable form of HMGB1 where all cysteines are replaced by serines, called 3S, to create a regenerative and non-inflammatory form of HMGB1. Following acute injury, treatment with 3S promotes muscle regeneration without exacerbating inflammation, by inducing the expansion of satellite cells and by mobilizing tissue-healing macrophages. Similarly, 3S administration accelerates liver regeneration after drug intoxication, bone healing after fracture and hematopoietic recovery following chemotherapy. Hence, the common regeneration responses to 3S indicate that the HMGB1/CXCR4 axis may be involved in the repair and regeneration of most tissues.

PRECLINICAL RESULTS:

Extracellular HMGB1 is present at high levels and undergoes oxidation in dystrophic patients and in mouse models of Duchenne Muscular Dystrophy (DMD) and Limb-Girdle Muscular Dystrophy type 2d (LGMD2D). Pharmacological treatment with the 3S variant improves functional performance, muscle regeneration and satellite cell engraftment in dystrophic mice, while reducing inflammation and fibrosis. Overall, the balance between HMGB1 redox isoforms dictates whether skeletal muscle is in an inflamed or regenerating state, and the non-oxidizable form of HMGB1 is a promising therapeutic approach to counteract the progression of the dystrophic phenotype and to potentiate combined treatments. **KEY FINDINGS:**



No genetic mutation restriction: preclinical data demonstrates the therapeutic properties of this drug candidate in different mouse models of muscular dystrophies; Drug with potent regenerative properties in multiple muscles and other tissues: treatment with this drug in preclinical models has shown potent regenerative properties in multiple tissues/organs (skeletal muscles, bone, liver); Synergic/complementary effects of the drug with current/future treatments: treatment with the 3S-HMGB1 promotes stem cell engraftment and expansion in dystrophic mice; High efficiency and ease of administration: a single systemic administration of this molecule per week for only 3 weeks is sufficient to observe functional improvement in muscles of dystrophic mice; No apparent toxicity: both in acute (a single administration) and chronic treatments (6 weeks treatment).

MARKET & COMPETITIVE CONTEXT

In 2020, the global Duchenne Muscular Dystrophy market size was USD 935 million, and it is expected to reach USD 9904.4 million by the end of 2027, with a CAGR of 42.1% during 2021-2027.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
HMGB1 variants and uses thereof	WO2014016417 US10626153	Granted	EP US

Compounds for use in Polycystic Kidney Disease

I.R.C.C.S. Ospedale San Raffaele
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Sector
Genetic disorders

Key Publications
Rowe et al, Nat Med, 2013
Chiaravalli et al, JASN, 2016;
Podrini et al, Comms. Bio., 2018.

Development stage
Preclinical Development

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

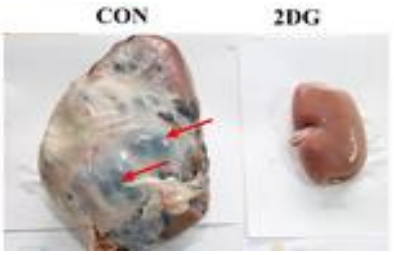
CLINICAL NEED

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most frequent rare monogenic disorder affecting approximately 3.5 to 14 million people worldwide. ADPKD is characterized by relentless development and growth of fluid-filled renal cysts, developing from any segment of the renal tubule and eventually causing progressive kidney enlargement. Pyelonephritis and cyst infections usually occur. When the majority of nephrons has been destroyed, renal function declines, typically after the fourth decade of life. Kidney failure requiring renal-replacement therapy (RRT; ie dialysis or kidney transplant) occurs in approximately 50% of patients and typically develops in the fourth to sixth decade of life. Tolvaptan is the currently available therapy able to retard disease progression; it's a vasopressin receptor antagonist that retards disease progression by lowering the levels of cAMP in the distal tubule and collecting duct of the kidney. However, inhibition of the vasopressin receptor is not curative. Disease progression is slowed down and not halted, therefore procrastinating the need of RRT. Among the several limitations related to the use of Tolvaptan, the major are: liver toxicity; potent diuretic effect, thus causing a sensible worsening in the quality of life of the treated patients; not effective in cysts of proximal tubule origin, nor in the cysts of the liver, often associated with ADPKD.

PRODUCT & TECHNOLOGY

The present technology is the use of the glucose analog 2-deoxyglucose (2-DG) in ADPKD. Studies of PKD mouse models have shown that PKD1 inactivation in vivo in the renal tubule results in increased glucose uptake and lactate production. Using a metabolomic approach, we have identified, both in cells from a murine model of PKD and from patients-derived ADPKD kidney, a new pathogenic ADPKD pathway involving defective glucose metabolism. Specifically, we showed that mutations in PKD1 results in enhanced glycolysis. In addition, experimental data indicate that the cells lining the cysts are more prone to uptake 2-DG than other cells and they are less effective in reprogramming their metabolism to use other energy sources

SOLUTION & BENEFITS



Glucose deprivation by 2-DG reduced proliferation and sensitized PKD1 mutant cells to apoptosis, suggesting that interfering with this pathway may be effective in slowing down cyst expansion in ADPKD. Moreover, 2-DG treatment ameliorated the proliferation rate and cystic kidney volume in a PKD mouse model. From a safety standpoint, it is worth noting that 2-DG has been tested in clinical trials in >200 patients as an anticancer drug (alone or in combination with chemotherapy), where 2-DG proved safe in the clinical setting. Among the reported side effects, the most common ones were attributable to hypoglycemic symptoms (somnolence, hunger, sweating) and were rapidly reversible with glucose administration. Dose limiting cardiac toxicity (QTc 3-degree elongation) was observed for chronic administration of the drug at doses higher than 45 mg/Kg, which are higher than the ones expected to be used in ADPKD patients (8 - 30 mg/kg).

MARKET & COMPETITION

A very large number of different signaling pathways were reported to be implicated in the processes underlying ADPKD pathogenesis and shown to be defective in ADPKD cystic epithelia, in particular the cyclic adenosine monophosphate (cAMP) and mammalian target of rapamycin (mTOR) pathways. In recent years, several compounds have been tested in preclinical studies based on original observations of dysfunctional pathways or biological processes identified. Meanwhile, global market regarding PKD therapy is made up of 56.555 past and ongoing deals for an overall value of 9 billion of dollars.

- Average yearly healthcare expenditure for ADPKD patients in Italy varying from €3,913.89 for non-dialyzed patients to € 45,059.62 for dialyzed patients.
- Tolvaptan global sales in 2017 were 604M$, forecasted to increase to 1.892M$ in 2024 (Globaldata).
- Considering the disease is progressive, chronic treatment has to be envisaged.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Compounds for use in polycystic kidney disease	US10478446 WO2014006093	Granted Pending	US EP

Duchenne Muscular Dystrophy (DMD) program

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Development stage
Preclinical

Key Publications
[1]Cossu et al. EMBO Mol Med 2015.
[2]Careccia et al. Sci Transl Med. 2021.
*Manuscript in preparation

Relevant Intellectual Property
[1]International patent application published as WO2007093412. Patent granted in US (US8071380). Patent pending in Europe

[2]International patent application published as WO2014016417. Patents granted in Europe (validated in 5 Countries) and in USA.

*Patent under filing.

CLINICAL NEED

Duchenne Muscular Dystrophy (DMD) is a devastating disease that occurs almost exclusively in males. Birth prevalence ranges from 1.6 to 3 per 10,000 live births (depending on different screenings). Loss of ambulation occurs at a median age of 12 and ventilation starts at about 20 years. There is international variation in use of corticosteroids, scoliosis surgery, ventilation and physiotherapy. The economic cost of DMD climbs dramatically with disease progression - rising as much as 5.7 fold from the early ambulatory phase to the non-ambulatory phase. DMD currently lacks an efficacious therapy and steroids are the only drugs that delay the progression of the disease but with serious side effects (Guiraud et al. 2015). On the other hand, new generation drugs (ASO for Exon skipping, small molecule for read-through premature nonsense stop codon), based upon correction of the mutated transcript, have not shown clear evidence of efficacy (McDonald et al. 2017; Lim et al. 2017), despite many trials and controversial market authorizations (Kesselheim & Avorn 2016).

SOLUTION & BENEFITS

To overcome the limitations that current approaches are experiencing within the treatment of DMD, and common to many other musculoskeletal disorders, we developed the below product pipeline enabling:

- A cell therapy approach, which proved to be safe in a Phase I clinical trial in 5 DMD patients. The approach is currently under optimization in order to develop an off the shelf, affordable product, for the creation of a universal donor mesoangioblast cell bank;
- A non-oxidizable form of HMGB1 (called 3S). Pharmacological treatment with the 3S variant improves functional performance, muscle regeneration and satellite cell engraftment in mouse models of DMD and Limb-Girdle Muscular Dystrophy type 2d (LGMD2D), while reducing inflammation and fibrosis;
- Matrin 3 (MATR3), a natural, physiologic, endogenous inhibitor of the transcription factor double homeobox 4 (DUX4). The aberrant expression of DUX4 is the main cause of FacioScapuloHumeral muscular Dystrophy (FSHD), for which there are no therapies. MATR3 binds directly to the DNA binding domain of DUX4 and blocks its activity

PRODUCT & TECHNOLOGY

Categories	Product	Field/Application	Development Stage
Cell therapy	Mesoangioblasts	Proved safety in 5 Duchenne Muscular Dystrophy (DMD) patients	PH I CT[1]
	Universal, Genetically Corrected Mesoangioblasts	- Human mesoangioblasts can be indefinitely expanded with the novel, proprietary, culture medium. - Off-the-shelf universal donor mesoangioblast cell bank. - Potential expansion to many recessive monogenic diseases of the mesoderm.	Lead opt.*
Biologics	Non-oxidable HMGB1	Targeting DMD regardless of the genetic mutation	In vivo PoC in DMD and limb-girdle MD[2]
	MATR3	- Safety of the approach (MATR3 fragment binds only to DUX4). - Versatile approach (MATR3 can be administered as biologics or gene therapy approach).	Lead opt/Animal test[3]
Enabler	HMGB1	Combination therapy with mesoangioblasts.	In vivo proof of concept[2]
Methods	Proprietary culture protocol for mesoangioblasts	Proprietary culture method	Research-grade reagents*

MARKET & COMPETITION

In 2020, the global Duchenne Muscular Dystrophy market size was USD 935 million, and it is expected to reach USD 9904.4 million by the end of 2027, with a CAGR of 42.1% during 2021-2027*. The propelling factors for the growth of the DMD treatment market include the rising disease burden, and increasing investments in biopharmaceuticals.

Exploiting an engineered minisacsin for gene therapy in the ARSACS preclinical model

I.R.C.C.S. Ospedale San Raffaele
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Sector
Gene Therapy

Key Publications
- Longo F, De Ritis D, et al. Neurology. 2021.
- Del Bondio A, JCI Insight. 2023.

Development stage
Preclinical Development

Business Model
Out-licensing for commercialization and/or sponsored research agreement with option rights.

The Technology
Gene miniaturization strategy for AAV vector delivery with enhanced brain penetration and transduction

CLINICAL NEED

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is the second most-common cause of recessive ataxia consisting in a neurodegenerative disorder characterized by early onset cerebellar ataxia and phenotypic variability, with spasticity, a pyramidal syndrome and peripheral neuropathy as leading symptoms.
ARSACS affects 2:100.000 individuals of all sexes and ages, with about 200.000 patients estimated in the US. ARSACS is caused by >200 described mutations in the SACS gene, encoding sacsin, a huge multimodular protein of unknown function mainly expressed in the Purkinje cells (PCs) of the cerebellum. Previous studies conducted in the Maltecca's lab indicate a role for sacsin in regulating the cytoskeleton which is fundamental for cellular functionality: in fact, cell and mouse models present severe alteration of cytoskeleton together with abnormal bundling and accumulation of neurofilaments Loss of function of the SACS gene indeed determines the loss of PCs in ARSACS patients and in the *Sacs-/-* mouse model which recapitulates human disease progression.
Currently, there is no cure or therapeutic option available to ARSACS patients: adeno-associated viral (AAV) vector-based gene replacement therapy could be ideal for this disease. Moreover gene replacement of mutated genes showed outstanding preclinical and clinical benefits for many inherited human diseases like ARSACS.

PRODUCT & TECHNOLOGY

Sacsin is a huge multimodular 520 kDa protein, made by different domains: a ubiquitin like domain (Ubl), three sacsin repeat regions (SRR), homologous to Hsp90 chaperone, a DnaJ domain homologous to Hsp40 and a nucleotide binding domain (HEPN) that suggest its eventual involvement in quality control mechanisms. Since AAV have a limited packaging capacity, the mini-gene strategy allowed the design of SACS minigene (minisacsin) containing only the necessary domains to safeguard sacsin function. The application of this solution determines a shortening of the length of the SACS gene: minisacsin fits in length with the packaging capacity of the AAV9 employed for the transduction of Purkinje cells and moreover the encoded protein keeps its functionality.
In addition the transduction of PCs has been performed employing the AAV-Se2 vector, which shows a distinct capsid variant determining a specific tropism for the neural tissue even upon systemic delivery: this feature significantly improved transduction efficacy of PCs.

SOLUTION & BENEFITS

The employment of AAV9 overcomes issues depending on the integration site and allowing to cross the blood brain barrier (BBB) in order to reach the therapy target area. In particular, a AAV9 variant has been developed in our Institute (AAV-Se2) able to target specific CNS areas, such as the of Purkinje cells.
Ex vivo experiments showed that PHP.eB mediated expression of minisacsin in Sacs KO PCs of the cerebellum after transduction is stable, safe and high leading to a recover of the bundle volume and a significant reduction of neurofilaments accumulation, which are typical features of the disease affecting PCs.
Moreover minisacsin expression in wild type PCs after transduction didn't affect the normal neurofilaments structure, as a sign of the safety of the strategy.

MARKET & COMPETITION

An engineered miniaturized version of the human sacsin protein delivered by adeno-associated viral vectors could be an ideal approach as gene replacement therapy for ARSACS patients: minisacsin expression is stable, high and safe, re-establishes its normal levels of expression for the rescue of the ARSACS phenotype affecting Purkinje cells of the cerebellum.
To date, no effective therapy exists for ARSACS patients: there is an urgent unmet medical need and the adoption of the mini-gene strategy to make SACS gene fit into the packaging capacity of the AAV represents a valid approach to recover the expression of SACS in ARSACS patients.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Coverage
Exploiting an engineered minisacsin for gene therapy in the ARSACS preclinical model	XXX	XXX

Technology

CytoChain: streamlining high-dimensional data handling in flow cytometry

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Sector
Technology

Key Publications

Manfredi F. et al. Eur. J. Immunol. 2021.

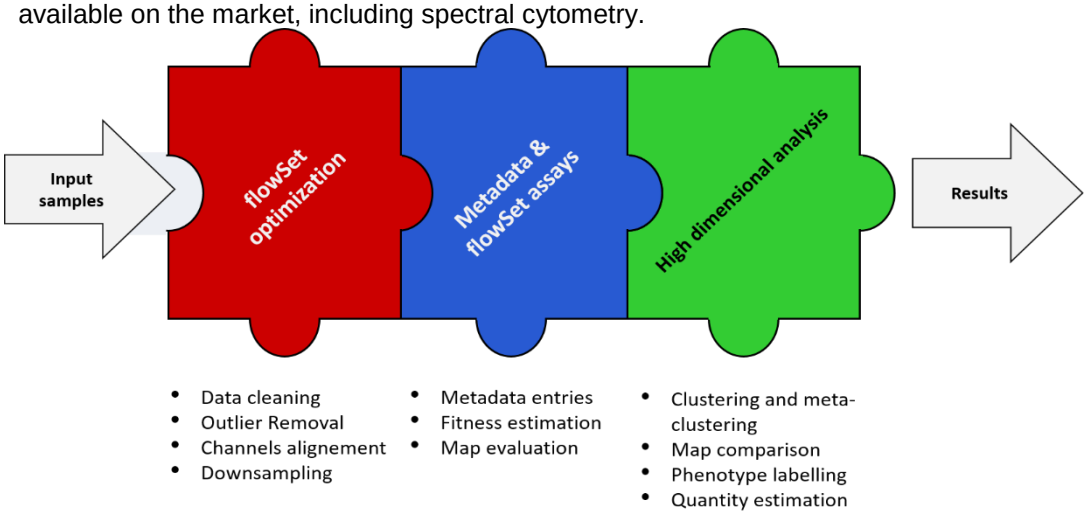
Development stage
Final version ready for testing. Some commercial library implementation are needed.

MARKET NEED

Polychromatic flow cytometry can capture the co-expressions of up to 50 surface markers at a single-cell level. Such technology produce large and complex datasets that benefit from data complexity reduction for their analysis. Different analytical platforms have been realized in recent years, but a customizable workflow able to integrate multiple analyses in a single environment is still lacking, or require several and complex operations to be carried out. To overcome current pitfalls, the scientist of san Raffaele Hospital developed cytoChain, a new application for high dimensional data handling, and defined and validated a novel analytical workflow.

PRODUCT DESCRIPTION

CytoChain is a comprehensive, modular application that can analyze and describe a complex flow cytometry dataset. The workflow can be divided in three phases: (I) FlowSet optimization, where the data are studied in their qualities and machine error is quantified and mitigated; (II) Metadata & FlowSet assays, where samples details are added as metadata and the best high dimensional analysis to be used estimated; (III) High dimensional analysis, where data undergo dimensionality reduction and high dimensional analysis. The software output consists of multiple graphs to aid in data comprehension, dissecting the effect of sample grouping. CytoChain is a standalone software that necessitates only a web browser to run. The sleek interface and pop-ups guide the user to handle the flow cytometry data according to the best possible workflow. CytoChain is intended to support the analysis of experiments generated with all the flow cytometers available on the market, including spectral cytometry.



COMPETITIVE ADVANTAGE

A complete software for high dimensional flow cytometry: currently available solutions require multiple platforms or multiple analytical runs to complete an analysis.
Easy to use: cytoChain has also been developed for users without special computer skills, the final data is presented graphically and is easily accessible.
Better quality and faster productivity: working in a single environment and the innovative solutions adopted make it possible to reduce times and improve the quality of the analysis.
Reproducibility: the strategies within by the software decrease the variability of the analyses performed.
Sensitivity: the proposed workflow improve the analytical depth of flow cytometry analysis, supporting new discoveries.
Modularity: the analysis is customizable according to the researchers' needs, as analytical modules can act independently.

MARKET & COMPETITIVE CONTEXT

The total market value of flow cytometry software is estimated at around 400/500 K€ per year. The users in the world exceed several thousand, with an annual growth rate of around 19% (CARG 2016-2021).

INTELLECTUAL PROPERTY

Actual version of cytoChain is used by several research groups in the frame of collaboration with the developer. The software is currently not publicly accessible. The commercial version will be protected by a software license.

Composite scaffold for osteochondral defects repair

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Sector
Technology, Orthopaedics, Tissue Engineering

Key Publications

1. Sosio et. Al Tissue Eng. Part A 2015, (21), 3-4
2. Crovace et al. Vet. Sci. 2019, 6(4), 90
3. Gervaso et. Al. J Biol Regul Homeost Agents 2016, (30), 24-31.

Development stage
Preclinical development

Business Model
Out-licensing for further development and subsequent commercialization.

CLINICAL NEED

In healthy subjects, articular cartilage and subchondral bone form a well-integrated system, with unique biomechanical properties, which provides the efficient transmission of high acting loads. This osteochondral core is frequently damaged by trauma and disease; as a consequence, the joint undergoes an osteoarthritis degeneration leading to severe pain, joint deformity, and loss of joint motion. Osteochondral defects are a common problem in both human medicine and veterinary practice. In the last years, several tissue engineering techniques have been applied to develop different kinds of osteochondral substitutes to overcome the scarce reparative properties of this tissue.

PRODUCT & TECHNOLOGY

Orthopaedic surgeons and engineers developed a biphasic scaffold for the regeneration of the osteochondral tissue, composed of collagen-1 and hydroxyapatite. This scaffold can be used as a cell-free implant or in combination with a source of cells. It shows good press-fit properties facilitating the filling of the defect and it is stable throughout long term regenerative applications (1). It has been validated in the swine model, in osteochondral defects of the trochlea where the scaffold was implanted both in the unseeded form and in association with autologous chondrocytes. Moreover, the scaffold was validated by implanting it in the medial femoral condyle (MFC) of the sheep model, in order to evaluate its potentialities in defects subjected to higher load-bearing activity (2,3). A novel version of the scaffold was tested in MFC of the sheep model, presenting columnar organization of the hydroxyapatite within the bony part of the osteochondral substitute. This new version presents also an important feature: the possibility to be cut and shaped before the implantation, based on the shape of the defect. Additionally, a growth factor releasing version of the scaffold will be produced and tested in vivo in order to potentiate the regenerative qualities.

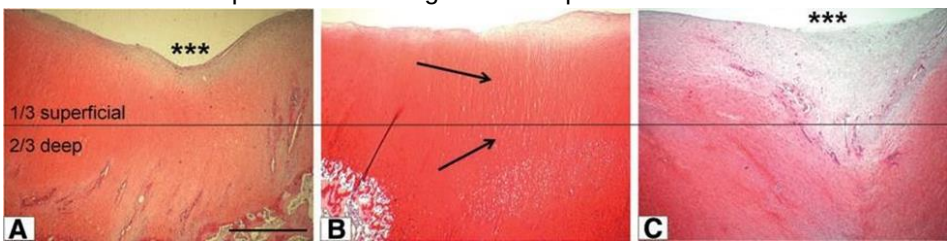


Figure 1. Glycosaminoglycans staining of the repaired tissue after 3 months. The cell free scaffold promoted a better regeneration of the chondral tissue (black arrows). *** indicates the center of the defect.

SOLUTION & BENEFITS

This scaffold presents significant competitive advantages with respect to the current commercial solutions:

- an interconnection zone between the upper collagen-1 layer (chondral phase) and the lower hydroxyapatite layer (bony phase), thus conferring a strong integration of the two materials and allowing for a better stability and integrity;
- an external thin layer made of collagen-1 that surrounds the whole scaffold, thus conferring good press-fit qualities to the composite and facilitating the insertion into the defect;
- an early regenerative potential of the chondral tissue;
- a cell free application, as this scaffold can be efficiently repopulated by cells deriving from the bone marrow or the surrounding tissues, as demonstrated by the in vivo experiments in the swine model.

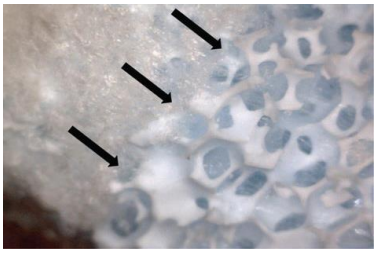


Figure 2. A detail of the integration between the collagen and the hydroxyapatite layers (black arrows).

MARKET & COMPETITION

The global scaffold technology market size was valued at USD 1.1 billion in 2020 and is expected to expand at a compound annual growth rate (CAGR) of 8.4% from 2021 to 2028. The orthopedics, musculoskeletal, and spine segment dominated the market for scaffold technology and accounted for the largest revenue share of more than 50% in 2020. It is estimated that nearly 34 million surgeries on the musculoskeletal system are performed in the United States annually. Hence, regenerative medicines are gaining momentum as they offer various lower-risk substitutes to allograft surgery.

INTELLECTUAL PROPERTY

Patent Title	Publication#	Status	Coverage
COMPOSITE SCAFFOLD FOR TISSUE REPAIR	WO2014184391	Granted	Europe USA

Liquid Biopsy Insertion Site sequencing (LiBIS-seq) from cell-free DNA

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

CLINICAL NEED

Gene Therapy (GT) has already shown to have the potential to revolutionize medicine and offer new treatment options for many otherwise incurable diseases. Once administered, it is crucial to clone-track the integration of the vector and monitor the fate of the engineered cells in the blood of the patient and therefore assess the safety and efficacy of the treatment.

Integration Site (IS) analyses are extremely useful to delineate the efficacy and safety of GT treatments, for the monitoring of patients treated with integrating vectors.

However, the limited number of available cells, and the impracticality of reaching cells in peripheral organs without the use of invasive procedures such as biopsies, provides only a partial snapshot of the dynamics of the engineered cells and reduces the predictive power of the safety readouts.

PRODUCT & TECHNOLOGY

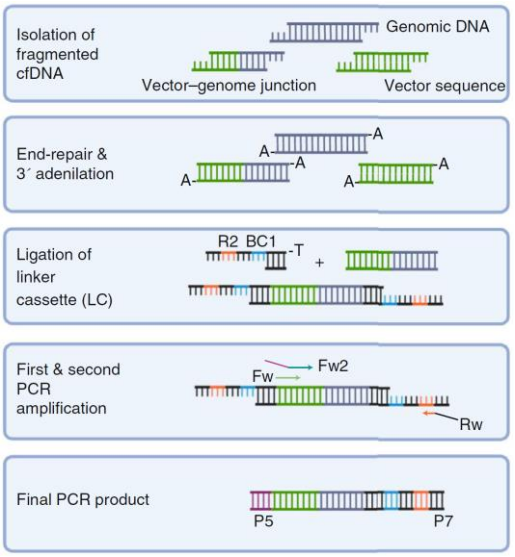
To address these issues, our researchers developed Liquid Biopsy Insertion Site sequencing (LiBIS-seq), a PCR technique optimized to qualitatively retrieve Integration Sites (ISs) from cell-free DNA (cfDNA) released into the bloodstream by dying cells residing in different tissues, and thus allowing timepoint monitoring of vector integration and genetically modified cells in patients receiving GT treatments. With LiBIS-seq, naturally sheared cfDNA fragments containing vector-cellular genome junctions, are molecularly tagged before PCR amplification and high-throughput sequencing, ensuring the sensitive and quantitative retrieval of ISs.

SOLUTION & BENEFITS

To date, IS analyses are widespread in clinical research to investigate the safety and efficacy of GT treatments, and are recommended by the U.S. FDA and EMA, for the monitoring of patients treated with GT using both viral and non-viral vectors.

IS performed with cellular DNA presents strong limitations, as it will not reach modified cells that travel to peripheral organs, resulting in a need of tissue biopsies when performed in vivo; these procedures are impractical and invasive, and offer a limited view of the impact of the therapy on the entire tissue, thus limiting the predictive value of the IS analysis.

LiBIS-seq addresses several of the limitations of IS analysis performed on cellular DNA; indeed, it is predictive, because it allows to detect malignant cells expanding in the blood and in peripheral organs, and it is informative of the efficacy and safety of the therapy, since it has been shown that the levels of cfDNA faithfully reflect the therapeutic response to treatment.



MARKET & COMPETITION

LiBIS-seq is a patented technology and represents a leap forward from currently available IS analysis technology that employs genomic DNA with its already-described limitations and issues.

Given the rich pipeline of GT products in the clinical pipeline for numerous therapeutic indications, LiBIS-seq could become an efficient and powerful tool, both in the hands of scientists and regulators, to better assess the safety and monitor the efficacy of GT products.

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Method for Analysis Insertion Sites	WO2020208206A1	Pending	EP USA CA

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Sector

Gene therapy

Key Publications

Cesana et al., Nature Medicine (2021)

Business Model

Out-licensing for further development and subsequent commercialization.

What we are looking for

Service Providers looking to implement this technology in their portfolio.

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Sector
Flow-cytometry
Immunology
BM transplantation
Gene therapy

Key Publications
- Lam, et al. Journal of Experimental Medicine. 2019.
- Capo, et al. Haematologica. 2020.
- Biavasco, Lettera, et al. Nat Comm. 2021.
- Montaldo, et al. Nature Immunology. 2022.
- Scala, et al. Nat Comm. 2023 (in press).
- Colantuoni, et al. Science Transl Medicine. 2023 (in press).
- Basso Ricci L, et al. Cytometry Part A, 2017.

Development stage
Clinical

Business Model
Out-licensing for commercialization.

Multiparametric Whole Blood Dissection (WBD): a one-shot comprehensive picture of the human hematopoietic system

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

TECHNOLOGY

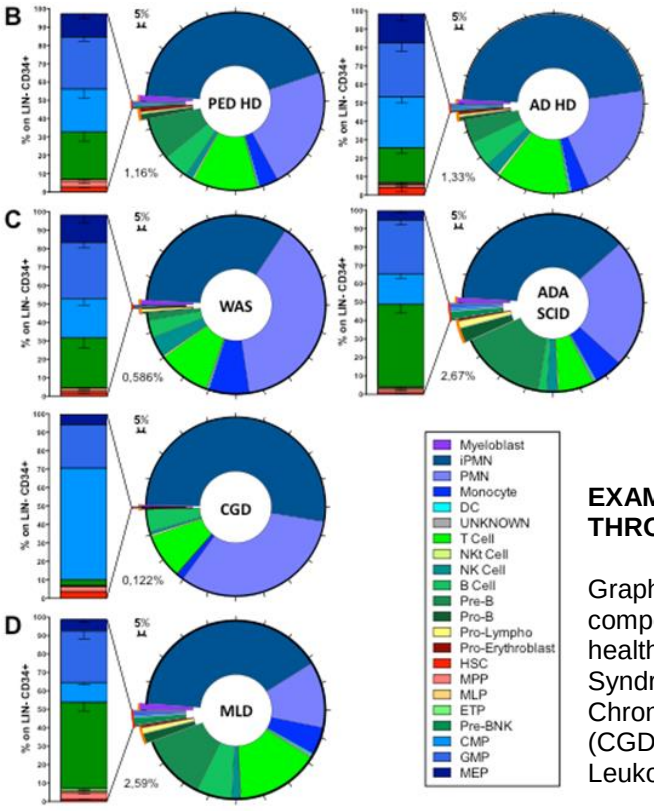
Flow cytometry protocol (kit), combining 17 surface markers, 1 viability cell marker and count beads. Starting from a limited amount of bone marrow (BM) or peripheral blood (PB) samples, WBD analyzes and quantifies at once and in a single test tube up to 23 different blood cell types, including human stem progenitor cell (HSPC) subpopulations and all major cell lineages at different stages of maturation.

APPLICATIONS

- **CLINICAL RESEARCH:** monitoring patients after transplantation/gene therapy; monitoring HSPC mobilization in patients and healthy donors for transplantation/gene therapy (quantity and quality of HSPC).
- **PRECLINICAL RESEARCH:** validation (efficacy and safety) of therapeutic approaches; testing novel delivery platforms.
- **BASIC RESEARCH:** study of hematopoietic reconstitution dynamics in xenotransplantation models; identification of progenitors involved in sustaining long-term hematopoiesis; Identification of novel cellular subsets.

COMPETITIVE ADVANTAGES

- **COMPREHENSIVE & SENSITIVE:** simultaneous quantification of 23 cell types.
- **FAST & CHEAP:** results in less than 1.5 hours, limited consumables and personnel.
- **POWERFUL:** limited amount of BM or PB samples, highly informative read-out.
- **STANDARDIZED & FLEXIBLE:** wide range of applications.



EXAMPLE OF RESULTS OBTAINED THROUGH WBD

Graphical representation of BM composition in 5 pediatric and 5 adult healthy donors (**B**), 6 Wiskott Aldrich Syndrome (WAS), 7 ADA-SCID and 1 Chronic Granulomatous Disease (CGD) patients (**C**), 6 Metachromatic Leukodystrophy (MLD) patients (**D**).

INTELLECTUAL PROPERTY

Patent Title	Publication #	Status	Coverage
Whole Blood Dissection (WBD)	WO2018/073267	Granted	US, EU, CA