

*Innovating Novel Clinical Protocols  
for Gamma Delta T, NK and Dendritic Cell  
Combination Therapy in Solid Tumors and HIV*

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# Disclosures

- Enochian Biosciences
- Pyrus Bio
- Frida Therapeutics

## Brief History of Adoptive Cell Therapies

- **The first historical example of adoptive cell therapy outside of HSCT was performed in 1986 with a positive clinical outcome in a patient with MRD of acute lymphoblastic leukemia (ALL), which was eventually named as donor lymphocyte infusion (DLI).** *(Slavin S, Or R, Naparstek E, Ackerstein A, Weiss L. Cellular mediated immunotherapy of leukemia in conjunction with autologous and allogeneic bone marrow transplantation in experimental animals and man. Blood 1988;72(suppl 1):407a)*
- **30-month old boy who was initially diagnosed with pre-B ALL and relapsed twice while on therapy. Received HSCT from his fully-matched sister. However, only one month after the HSCT, the patient presented with a hematological and metastatic relapse with multiple CNS solid tumors.**
- **To induce a stronger graft-versus-leukemia (GVL) effect, he was given peripheral blood lymphocytes harvested from his donor.**
- **Following DLI, the patient developed grade II GVHD, which was responsive to corticosteroids. Within two weeks after the DLI infusions, patient's masses started to decrease in size and his peripheral blood and bone marrow (BM) morphology exhibited 100% donor chimerism.**
- **Patient is still alive and healthy today.**

## Brief History of Adoptive Cell Therapies

- Following his case, many oncologists around the world reported the effectiveness of DLI in patients with cancer cells resistant to conventional chemotherapies.
- Subsequently, it became a standard therapy for recurrent hematologic malignancies and paved the road for the other potential uses of allo-reactive immune cells for recurrent cancers that are resistant to conventional therapies.
- After reports of clinical successes of DLI and other adoptive cell therapies such as lymphokine activated killer (LAK) cells, the myeloablative preconditioning regimen used prior to allogeneic HSCT also evolved into reduced intensity conditioning (RIC) which led to the development of non-myeloablative HSCT.
- In subsequent years, GVL and GVT were successfully employed outside of the context of allogeneic HSCT by using intentionally HLA-mismatched NK and T cells following a mild preconditioning with low dose lympho-depletive chemotherapeutics in patients with hematological malignancies and metastatic solid tumor cancers.



## Factors Affecting Clinical Outcome

- **Stage of disease, or more importantly, timing of the cell therapy—→ MRD**
- **Immunological baseline of the patient (immune perturbations caused by cancer, side effects of chemotherapy, immunotherapy, and/or radiation therapies)**
- **Preconditioning regimen (lympho-depletive vs lymphosuppressive (-> secondary “vaccine” effect)**
- **Persistence and continued activation of donor cells in vivo (repeat doses, cytokine administrations after cell therapy, checkpoint inhibitors, gene modifications on donor cells, etc)**
- **Potential tolerogenic effects of cell therapy (downregulation of PGE2 and FOXP3 pathways to reduce Treg differentiation and tolerogenicity)**
- **Possibility of maintenance immunotherapies to keep the host immune system working against cancer long after cell therapy (checkpoint inhibitors, DC vaccines, etc)**

## Modern use of NK cells- Clinical protocol example

Lympho-depletive chemotherapy (Flu or Cy)



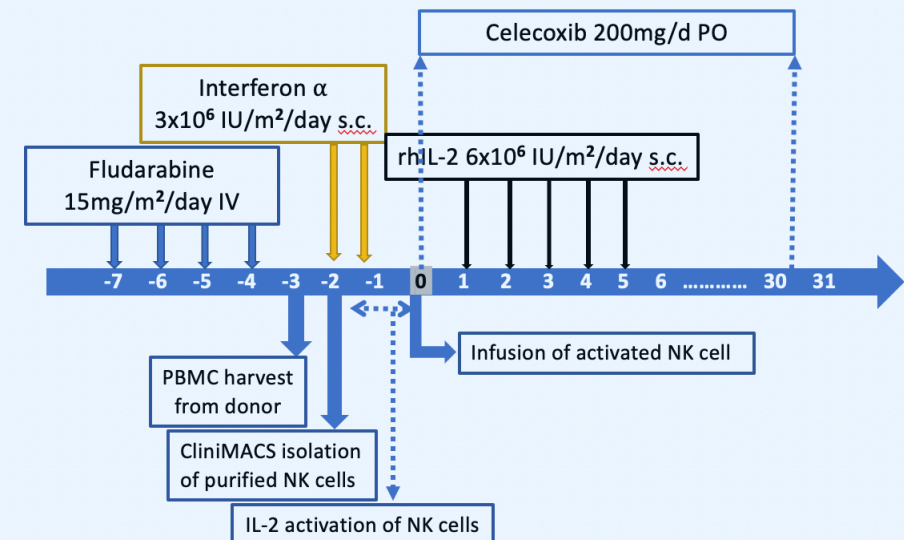
Intentionally mismatched ex vivo activated NK cell infusion



In vivo administration of rIL-2 for continuous cell activation



COX-2 inhibitor to reduce Treg differentiation



# Past Clinical Outcomes\*

Patients Treated with Allogeneic NK Cell Therapies			
Metastatic Solid Tumors	# of patients	Hematological Malignancies	# of patients
Colorectal cancer	1	Multiple myeloma	2
Pancreatic cancer	2	CLL	2
Ovarian cancer	2		
Breast Cancer	4		
Renal cell carcinoma	2		

## Survival of Patients (Solid and Hematological Malignancies)

	No. of Patients	Survival in months
Alive-PFS	10	>66 CRC, >74 PanC, >90 BC, >68 BC, >32 RCC, >102 OvC, >92 MM, >82 MM, >110 CLL, 48 CLL
Alive-SD	2	>22 BC, >18 RCC
Alive-PD	1	>12 OvC
Deceased	2	1-6 months

PFS= progression-free survival, SD= stable disease, PD= progressive disease, CRC =colorectal cancer, PanC =pancreatic cancer, BC= breast cancer, OvC= ovarian cancer, RCC= renal cell cancer, MM= multiple myeloma, CLL= chronic lymphocytic leukemia

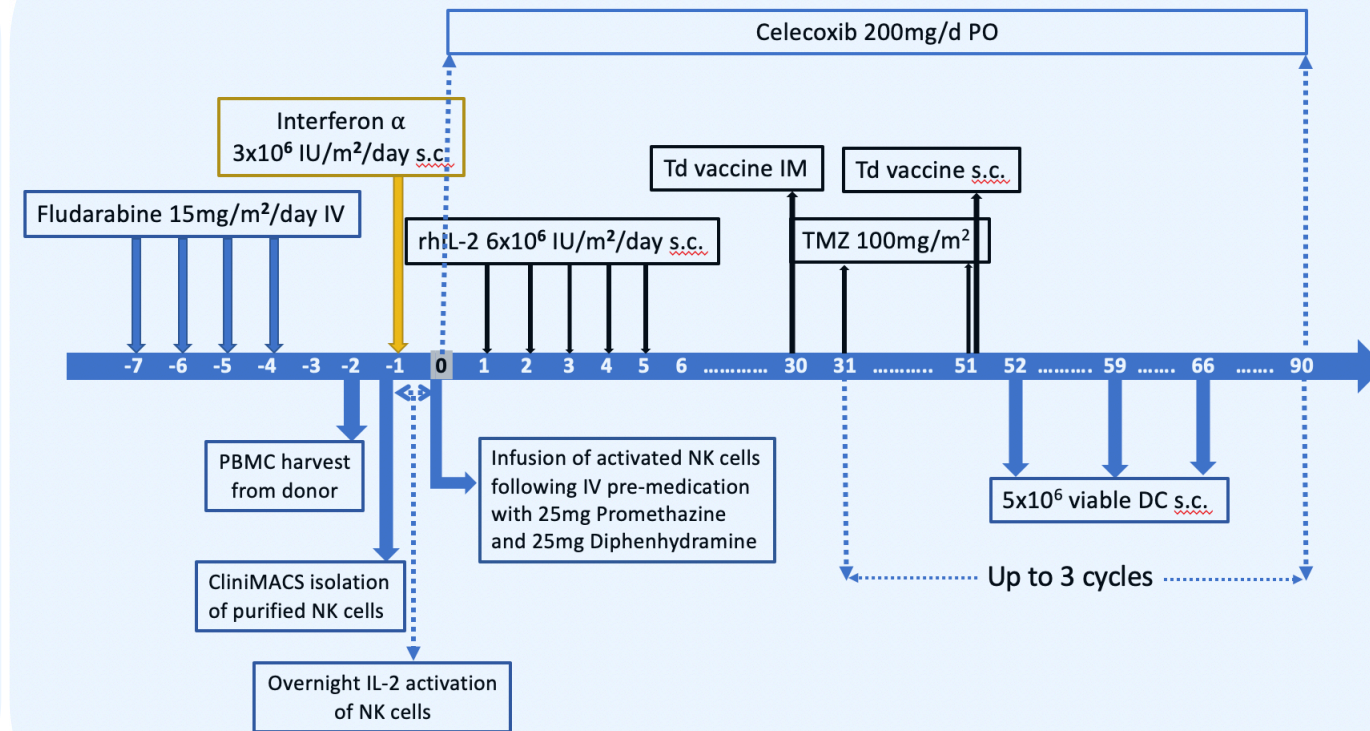
## Treatment Toxicity using WHO Criteria

	Grade I	Grade II	Grade III	Grade IV
Hemoglobin	9	4	0	0
WBC	10	3	2	0
Neutrophils	8	4	1	1
Platelets	5	4	2	0
Edema	3	1	0	0
Pericardial Effusion	2	1	0	0
Fever	4	9	2	0
Allergy/Hypersensitivity	6	2	0	0

\*Gumrukcu et al, Seraph Research Institute and collaborators' single-patient studies or treatment INDs (2014-2022)

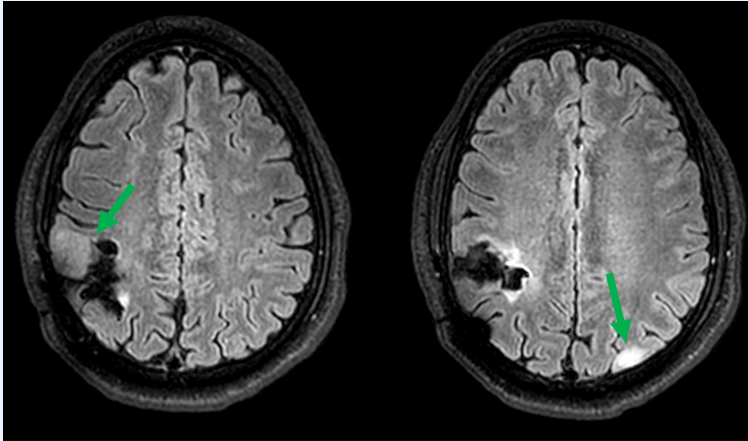
# Clinical Example 1 – Patient History

- 36-yo male
- Recurrent bilateral Glioblastoma (GBM) with subtotal tumor resection (July 2019)
- Standard therapy for recurrent GBM -> Chemotherapy (temozolomide) and radiation, 6 months
- Mean time to tumor recurrence in gross total resection - > 9.3 months
- Overall survival -> 11.1 months
- Prognosis dimmer in subtotal resection with larger residual lesions.
- Patient received one cycle of radiation and temozolomide (Aug 2019) and opted out to standard therapies due to expected poor outcome.
- Patient received haplo-identical mismatched NK therapies (Sept 2019, Oct 2019)
- Patient received allo-DC vaccine for 3 cycles (Oct 2019 to Jan 2020)

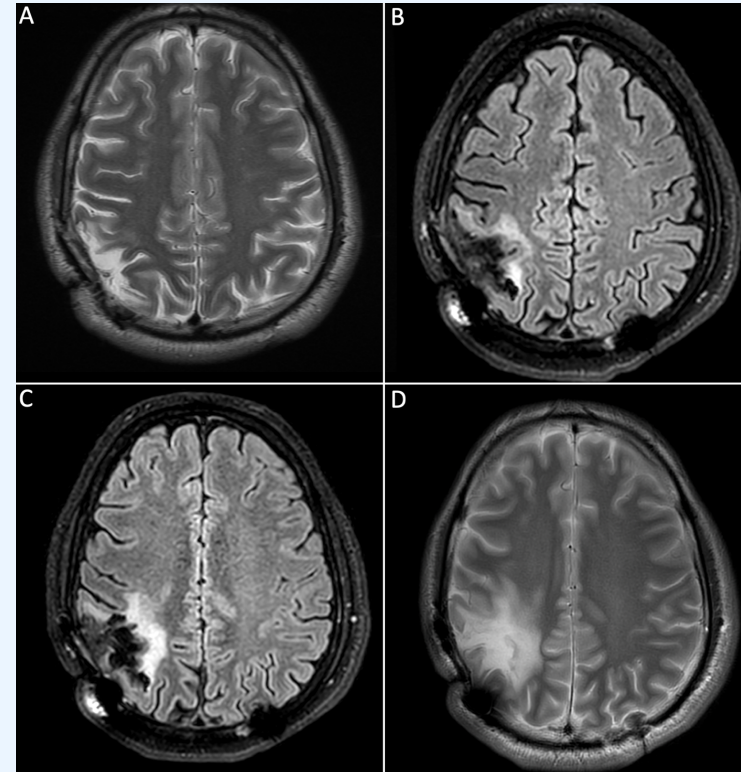


# Clinical Example 1 – Clinical Outcome

- No visible residual lesion in perfusion MRIs starting Oct 2019
- Complete remission: 29 months since remission achieved, 26 months since the end of therapy
- As of 12 months of remission, tumor-specific (CMV-pp65-specific) T cell clones persistent in the patient, with no signs of exhaustion



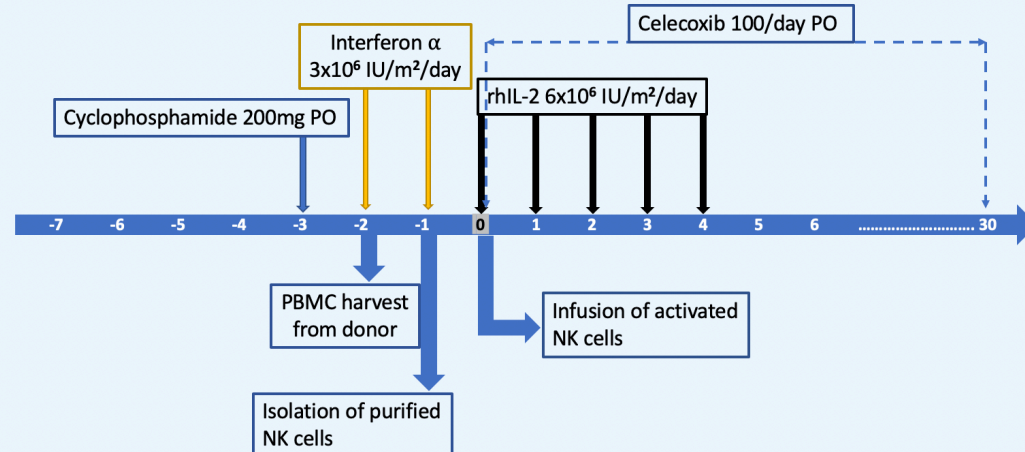
Top image: Recurring residual tumors  
Right image: (A) 3-mo, (B) 6-mo, (C) 12-mo, (D)  
15-mo follow up MR images.





## Clinical Example 2 – Patient History

- 8.5-yo female
- Optic pathway glioma
- History of multiple chemotherapy regimens
- History of multiple surgeries
- Last surgery in Feb 2021
- Patient received haploidentical (parent) NK cells following low-dose directed radiation therapy



2014 to 2020

Multiple lines of chemotherapy and partial resection surgeries

February 2021

Partial resection and cyst drainage due to severe clinical symptoms including blindness (limited light perception, no color perception)

August 2021

MRI study shows progression of disease

September 2021

NK cell therapy from related donor

November 2021

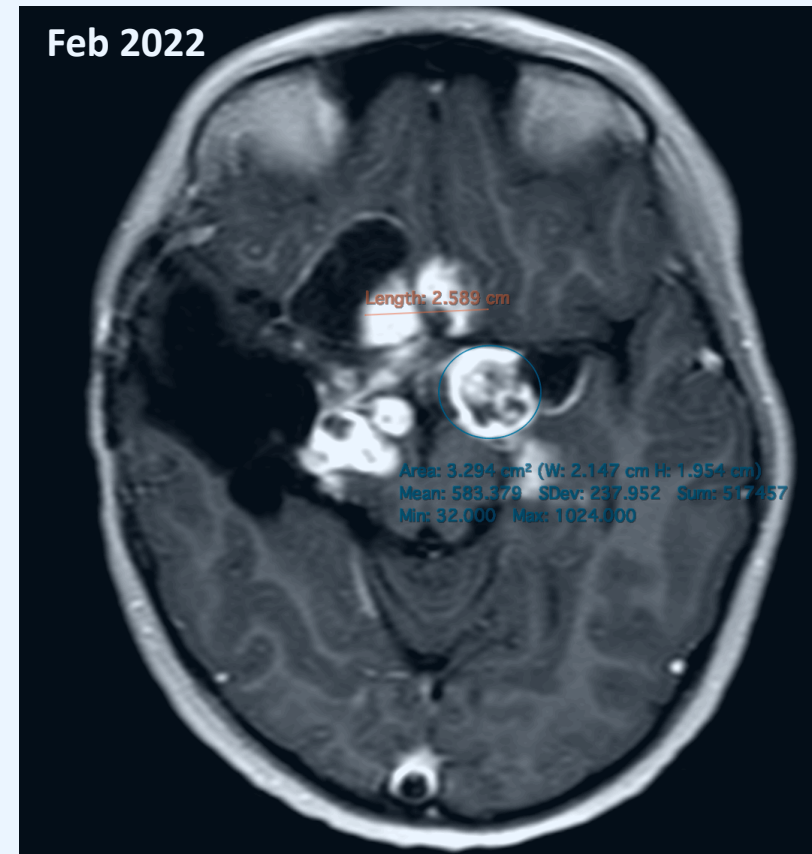
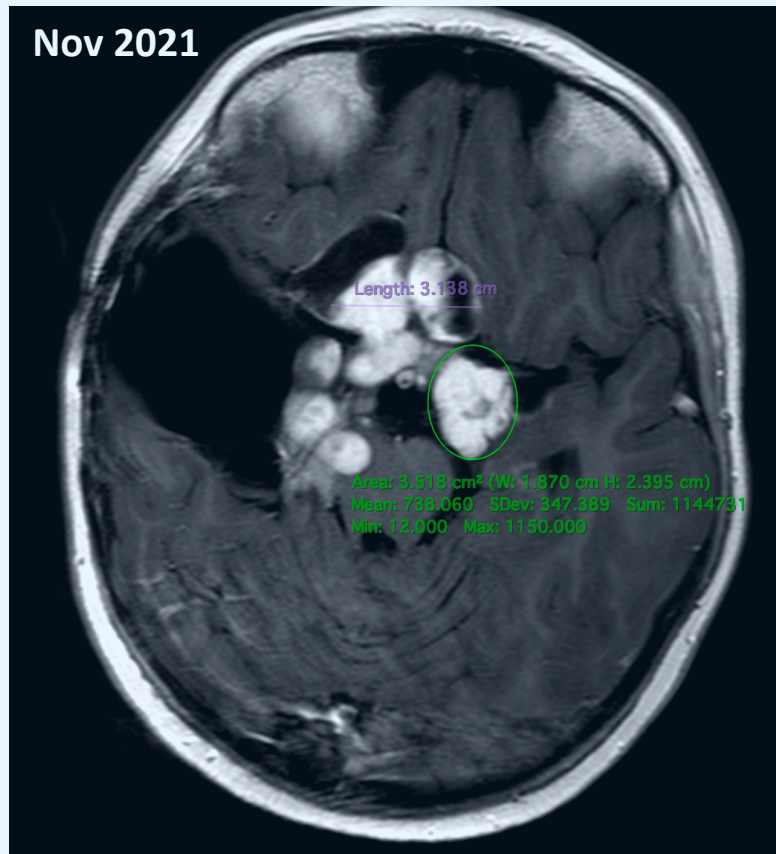
Stable disease

December 2021

NK cell therapy from related donor  
Subsequent DC vaccines from the same donor

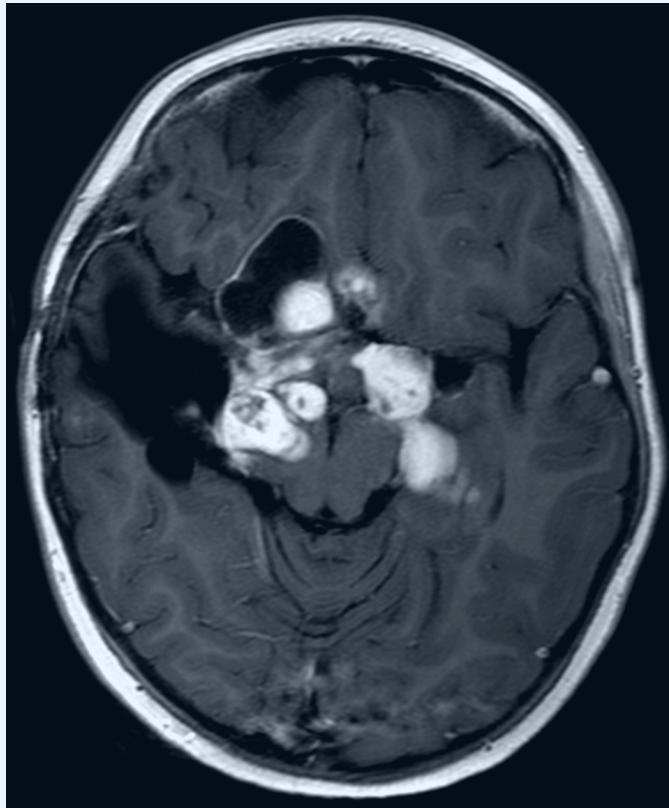
## Clinical Example 2 – Clinical Outcome\*

- Reduction in cystic formation around the tumor. Reduction in tumor size and viability by perfusion MRI and spectroscopy
- Clinical improvement in muscle coordination.

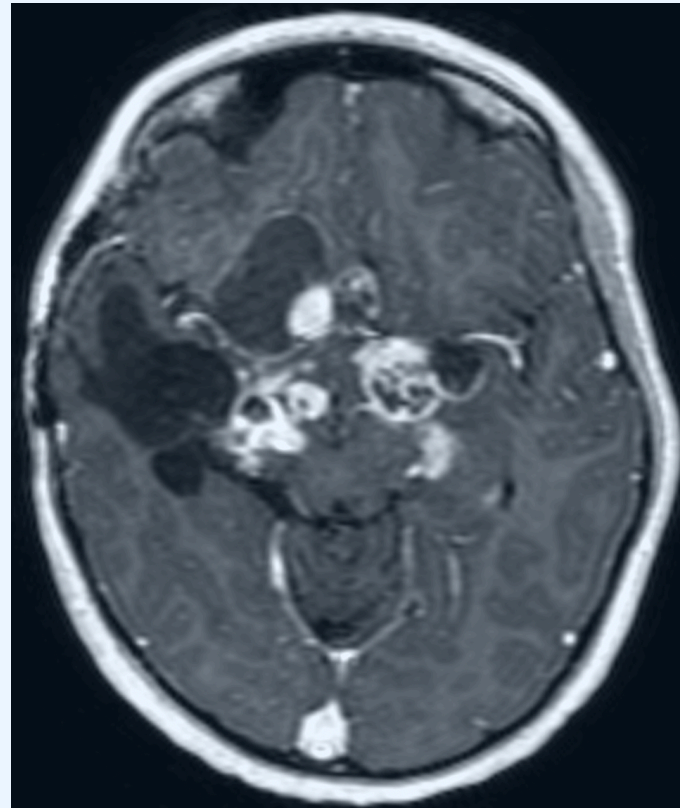


## Clinical Example 2 – Clinical Outcome 3

- Clinical improvement in vision and visual acuity. (light perception improved, color and shape perception developed first time)
- Reconstitution of endogenous lymphocyte and NK populations including improvement in function.



**Nov 2021**



**Feb 2022**



# Clinical Example 2 – Clinical Outcome\*

**Feb 2021**

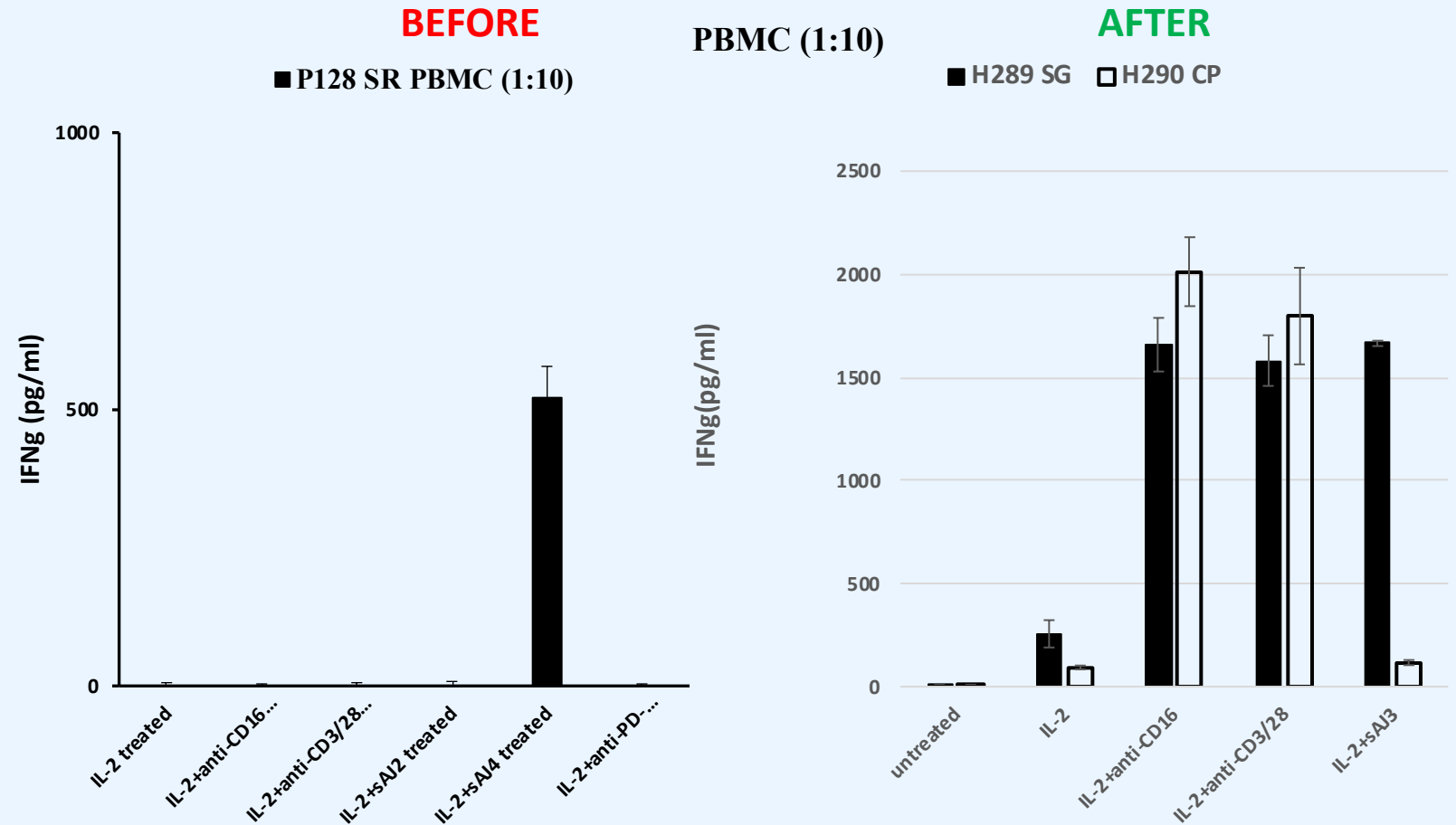
124e6 PMBCs in 20ml whole blood

	P128 SR	H229 TC
NK	4.28%	9.43%
CD4	28.3%	36.1%
CD8	19.5%	31.0%
CD19	36.9%	9.77%
CD14	11.2%	20.2%
total	100.18%	106.5%

**Feb 2022**

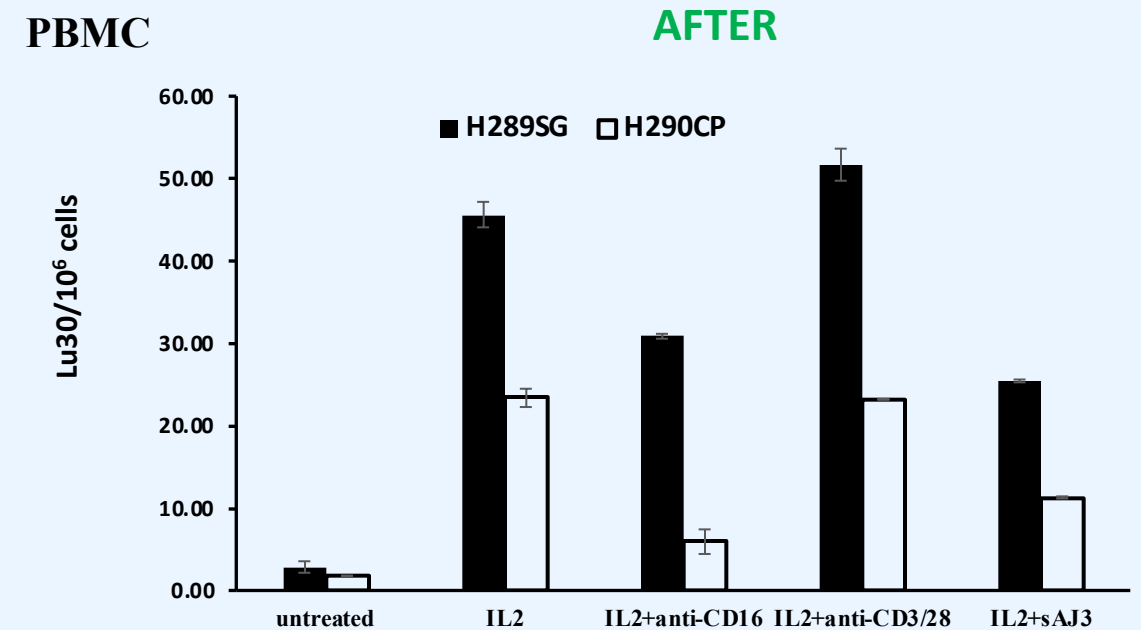
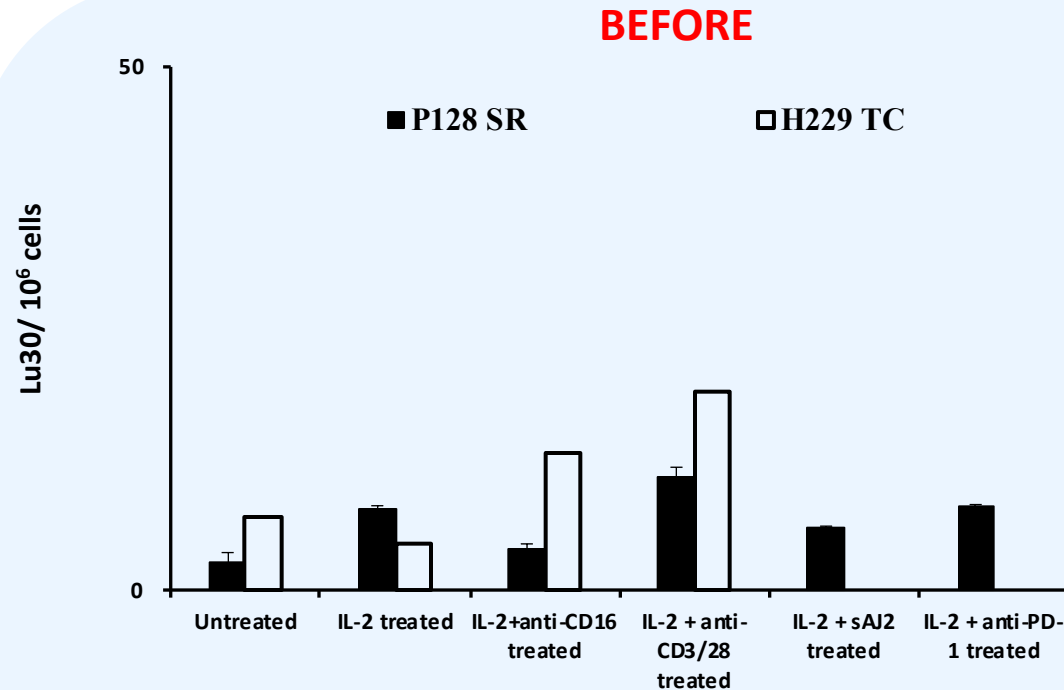
210e6 PMBCs in 20ml whole blood

	H289 SG	H290 CP
NK	5.81%	10.5%
CD4	53.7%	39.0%
CD8	27.7%	15.6%
CD19	10.2%	18.9%
CD14	5.87%	14.8%
total	103.28	98.8



IFNγ ELISA on PBMCs (1:10) before and after NK therapies. PBMCs were cultured alone, with IL-2, with IL2+antiCD16 ab, IL-2+sAJ2 or IL-2+sAJ3.

## Clinical Example 2 – Clinical Outcome\*



Chromium release assay results from pre- (Feb 2021) and post-NK (Feb 2022)

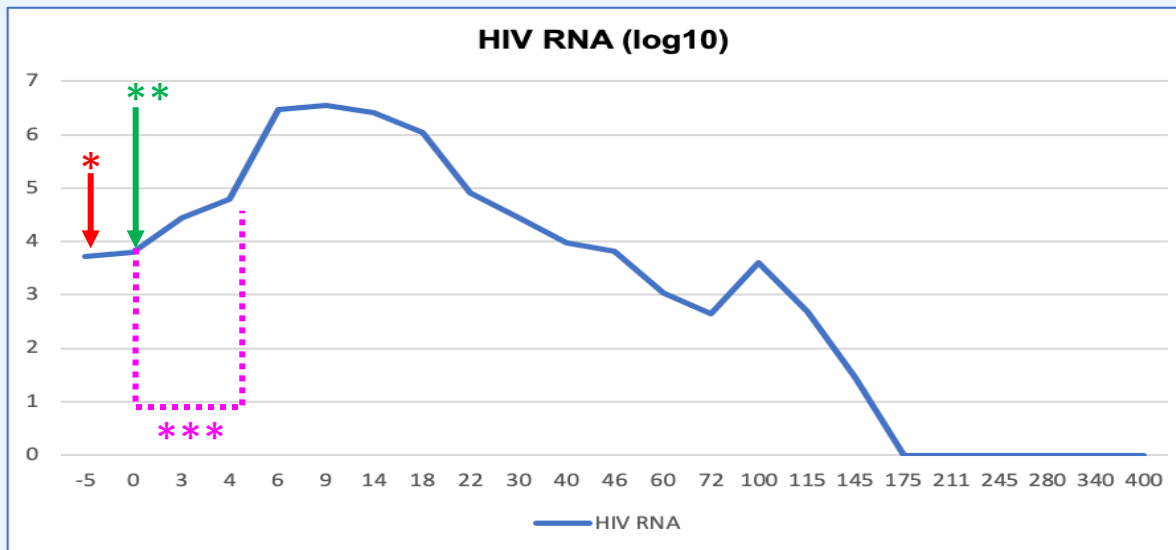
IFNg (NK) pg/ml	untreated	IL-2	IL-2+aCD16	IL-2+sAJ3	IL-2+sAJ4
2/21 (post-surgery)	0	0	N/A	N/A	14.1615
10/21 (post-NK#1)	7.84	4.80	227.55	N/A	89.85
2/22 (post-NK#2)	35.25	59.01	399.98	322.21	461.51

IFNg (CD8 1:5)	untreated	IL-2 treated	IL-2+anti-CD3/28	IL-2+sAJ3
2/21 (post-surgery)		0.00	55.07	8.73
10/21 (post-NK#1)		10.44	72.74	17.56
2/22 (post-NK#2)	9.62	11.68	1887.40	941.00

IFNg ELISA on NK and CD8+ cells before and after NK therapies. The cells were cultured alone, with IL-2, IL-2+anti-CD3/CD28 ab, or IL-2+sAJ3

## Clinical Example 3

- 54-yo male
- HIV since 1986
- History of monotherapy, dual therapy, etc
- Uncontrolled low viremia under combination ART (VL ~2,000 copies)
- Low dose lympho-suppressive pre-condition regimen for 3 days (day -5 to day -2)
- $25 \times 10^6/\text{kg}$  NK cells and  $5 \times 10^6/\text{kg}$  gd-T cells on day 0 from a partially mismatched healthy donor
- Low dose in vivo rIL-2 administration for 5 days post cell infusion
- Discontinued ART on day -5 and remained off for duration of study
- Patient experienced grade II and III adverse reactions (not due to cell therapy but due to rIL2 treatment)



\* Pre-conditioning chemotherapy  
Flu 15mg/m<sup>2</sup>/d x 3 days

\*\* Cell infusion

\*\*\* rIL-2 6mio IU/m<sup>2</sup>/d x 5 days

# Conclusion

- **Allogeneic primary NK therapies are safe, and can be very effective when they are administered at a favorable time (MRD stage in cancer patients, controlled or partially controlled viremia in HIV) and when the clinical protocol is tailored to overcome certain shortcomings of cell therapies.**
- **These therapies still have limited effects on heavy tumor burden patients.**
- **Follow up/maintenance immunotherapies can be very helpful for maintaining immunological control of the disease (checkpoint inhibitors, DC vaccines, etc)**
- **We still face large-scale manufacturing challenges using primary NKs but promising clinical results are motivating to solve such issues.**

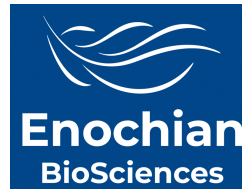
# Thank you!



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