3rd International Meeting
ADVANCES IN MALIGNANT LYMPHOMA: MAXIMIZING THE BASIC-TRANSLATIONAL INTERFACE FOR CLINICAL APPLICATION
IN COOPERATION WITH THE INTERNATIONAL CONFERENCE ON MALIGNANT LYMPHOMA (ICML)
June 23-26, 2022 | Westin Copley Place | Boston, MA
AACR American Association for Cancer Research
FINDING CURES TOGETHER

Development of Nicotimanide Enhanced NK cell Therapeutics for Lymphoma

Veronika Bachanova, MD, PhD
Professor of Medicine
University of Minnesota

HEALTH
UNIVERSITY OF MINNESOTA

Veronika Bachanova, MD

I have the following relevant financial relationships to disclose:

Employee of: none

Consultant for: Karyopharma, ADC, Astra Zeneca, Gamida Cell

Speaker's Bureau for: none

Grant/Research support from: Incyte, Gamida Cell, BMS, FATE Therapeutics,

Stockholder in: none

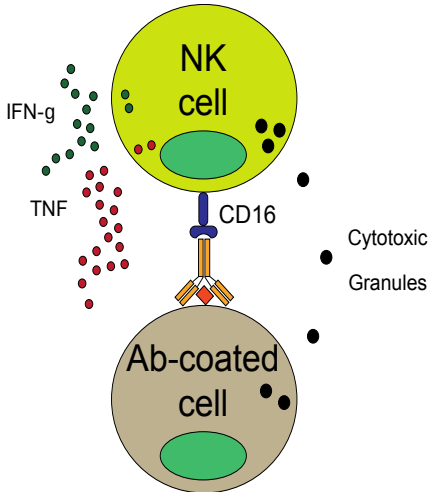
Honoraria from: none

- and -

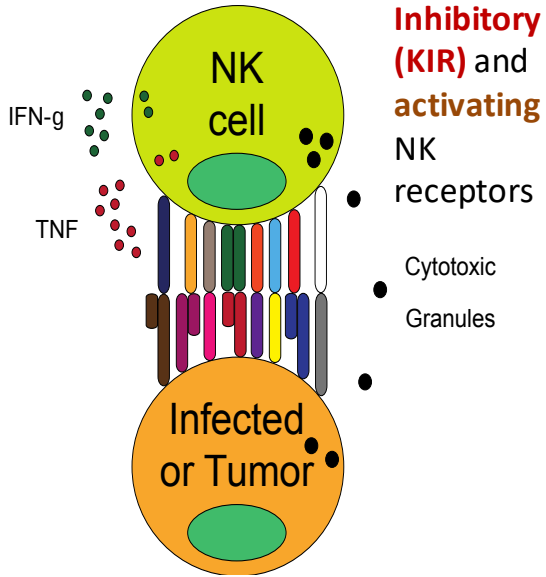
My additional financial relationship disclosures are: none

NK Cell Biology Informs on Clinical Strategies to Augment NK cell Function

Antibody-Dependent Cellular Cytotoxicity (ADCC)

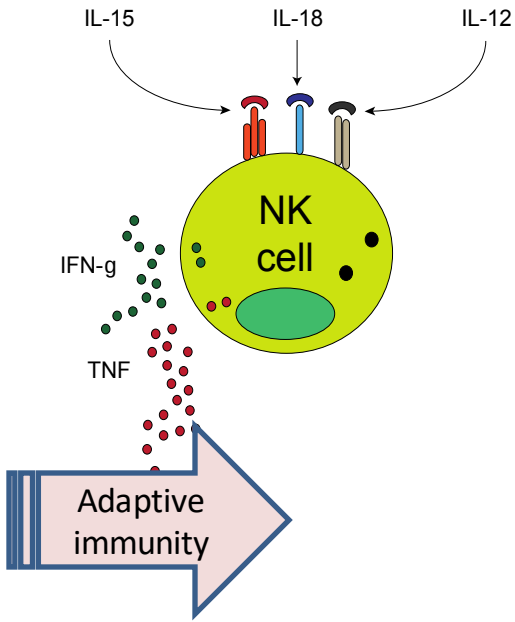


Natural Cytotoxicity



Inhibitory (KIR) and **activating** NK receptors

Cytokine Stimulation



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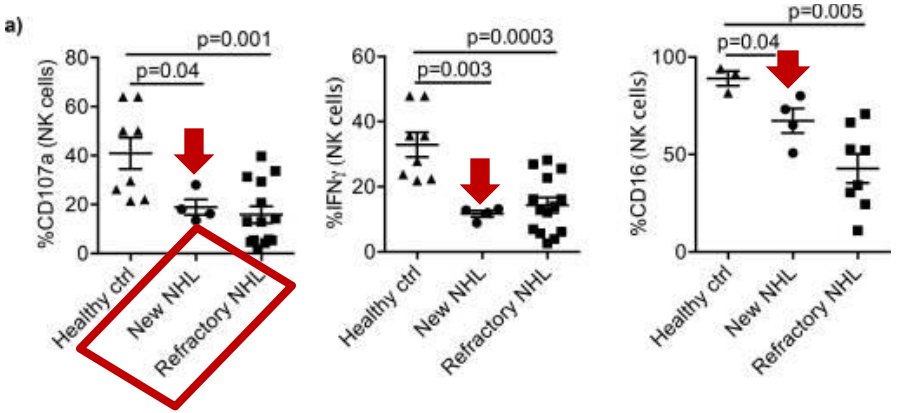
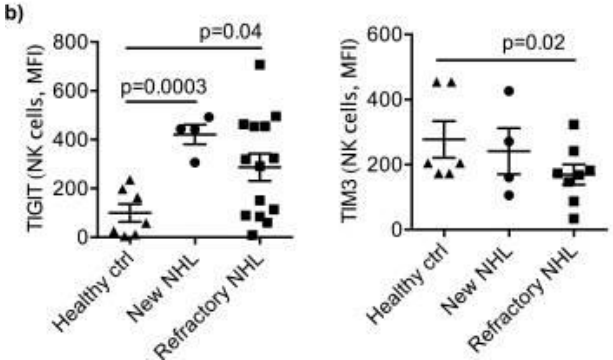
Progressive Decline of NK cell function in patients with relapsed/refractory lymphoma

NK cells have impaired killing, IFNg production and lower CD16 expression compared to healthy controls

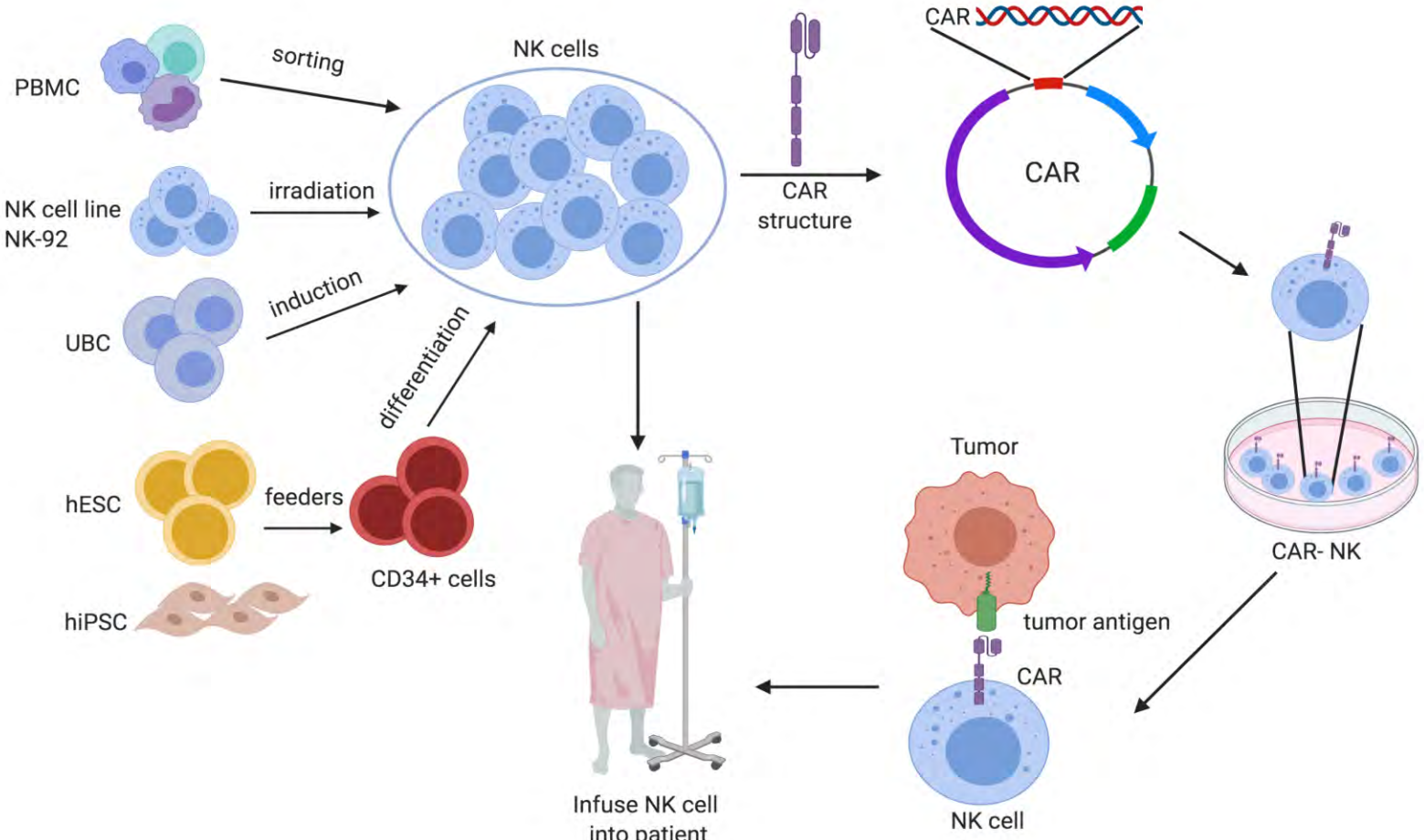


NK cell have altered expression of immunosuppressive receptors TIGIT and TIM3



Bachanova V, Cancer Immunol Immunother. 2018 Nov;59(11):1739-44.

NK Cell Sources For Cell Therapy



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Partially HLA matched related donor

Peripheral Blood apheresis

NK-enriched

Eligible patients: Poor prognosis with R/R AML and NHL

Cy 60 mg/kg x 1 day
Flu 25 mg/m^2 x 4 days

CD3 depletion (+added CD19 depletion)
IL-2 co-incubation overnight

HLA- haplo matched NK cells
2-8 x 10^7 MNC/kg
+ RITUXIMAB

IL-2. 10 MU SQ QOD x 6

Miller et al, Blood 105:3051-3057, 2005 pioneered for AML , Bachanova et al. Blood 2014 AML cohort

Bachanova et al, Cancer Immunol Immunother. 2010

Safety and Limitations of PB Allogeneic Donor NK infusions

1st allogeneic NK cell trial for lymphoma (NCT01181258) launched in 2008
First 6 patients reported in 2010
Subsequent 16 patients were enrolled

- No serious infusion toxicity
- No GVHD
- No Cytokine Release Syndrome
- No Neurotoxicity

Clinical efficacy in R/R lymphoma with ORR 28%
Low but we proved the concept !

Haplo PB NK cells persisted in blood for up to 7 days

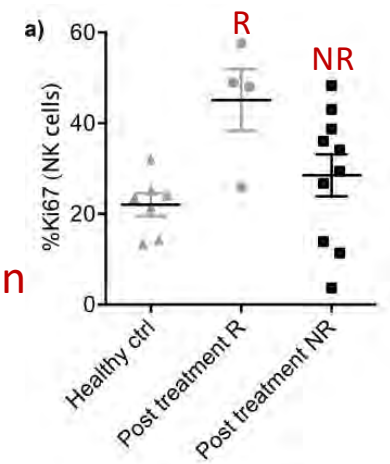
Regulatory T cells (host) expanded in some patients due to high dose IL-2

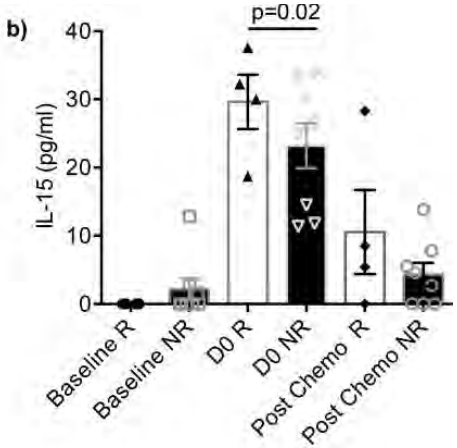
Bachanova V, Cancer Immunol Immunother. 2010 Nov;59(11):1739-44.

Responders experienced higher NK cell proliferation and endogenous IL-15 levels compared to non-responders

R responder
NR non-responder

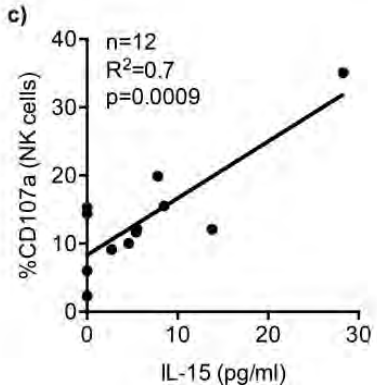
NK proliferation





Endogenous serum IL-15 levels

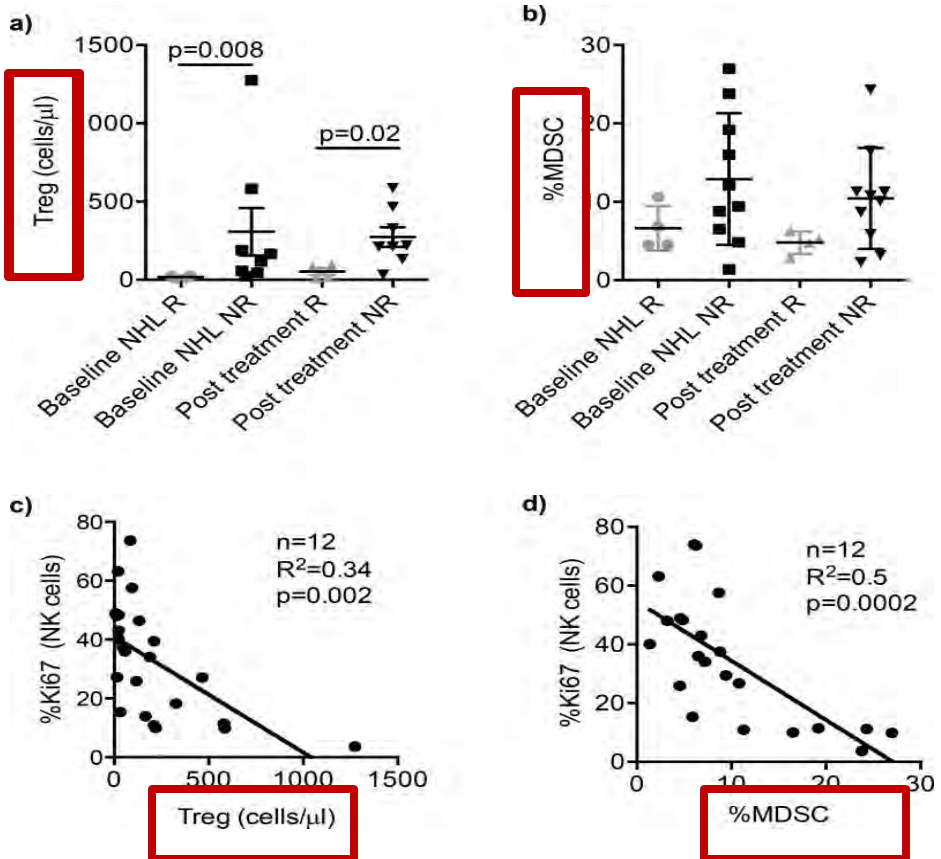
Endogenous serum IL-15 levels correlated with NK cell cytotoxicity



Bachanova V, Clinical Cancer Research, 2018; **67**, 483–494

Haploidentical NK cells induced remissions in NHL patients with low levels of immune-suppressor cells

R responders
NR non-responders



Bachanova V,
Clinical Cancer Research, 2018; **67**, 483–494

Opportunities and What Did we Learn?

- Blood derived NK cells exhibit short-term persistence in vivo
- Hostile immune environment inhibits NK cell function
- Wide variability of PB NK cell product content (NK cells content ~40%; other cells: monocytes, B-cells, T cells <5x 10*5)
- Limited NK cell dose due from PB
- Suboptimal clinical efficacy

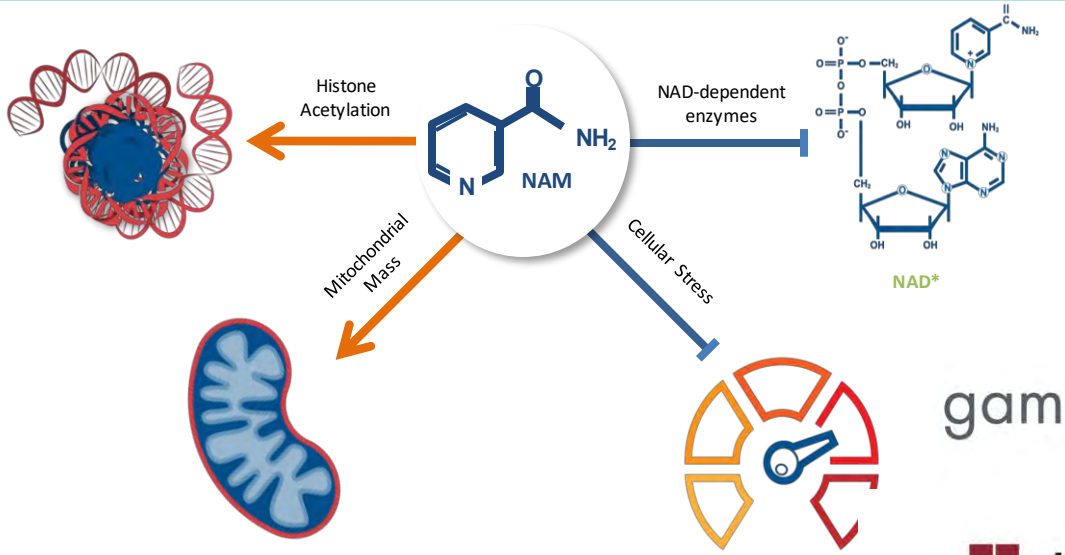
Barriers to overcome

Metabolic Re-programming of Hematopoietic Cells with Nicotinamide

Nicotinamide can expand any cell type (developed to expand CD34 cells from UCB)

Importance of NAM

- is a potent allosteric inhibitor of NAD–dependent enzymes
- Plays a key role in metabolic reprogramming of cells
- Is a master regulator of NAD-related signaling pathways
- Transcriptional regulator
- **Preserves cellular functionality and phenotype during in vitro expansion**
- **Enhance the Number of Cells in Culture**



gamida cell

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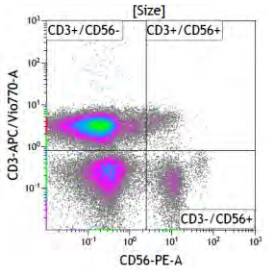
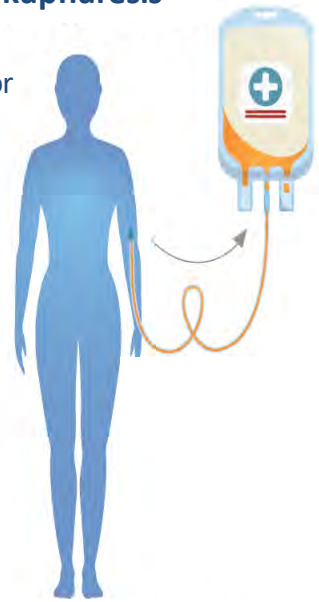
Nicotinamide – induced PB donor NK cells (GDA-201)

Leukapharesis

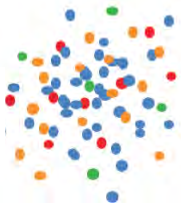
Donor

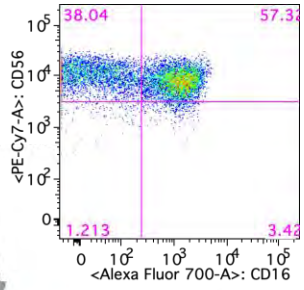




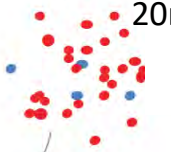
Donor NK cells obtained from HLA-haploidentical or mismatched related donor

CD3 depletion at seeding



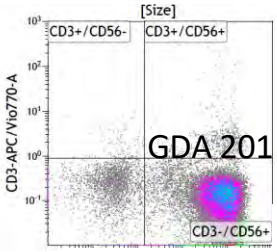


20ng/ml
IL-15 + NAM co-culture



14 day culture
NK cells > 98%
T cells < 0.5%

GDA-201



Highly functional NK cells:
40-fold increase in NK cells
~50 - 100 billion, purity >98%

gamida cell

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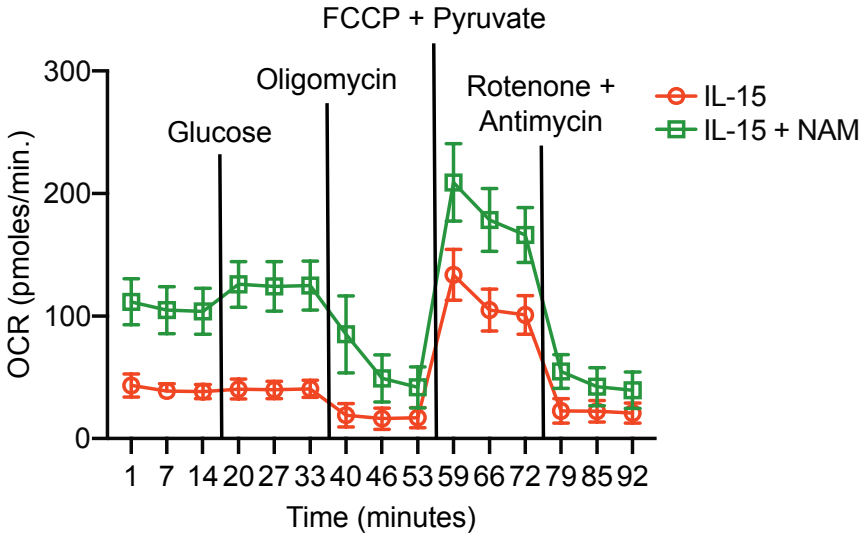
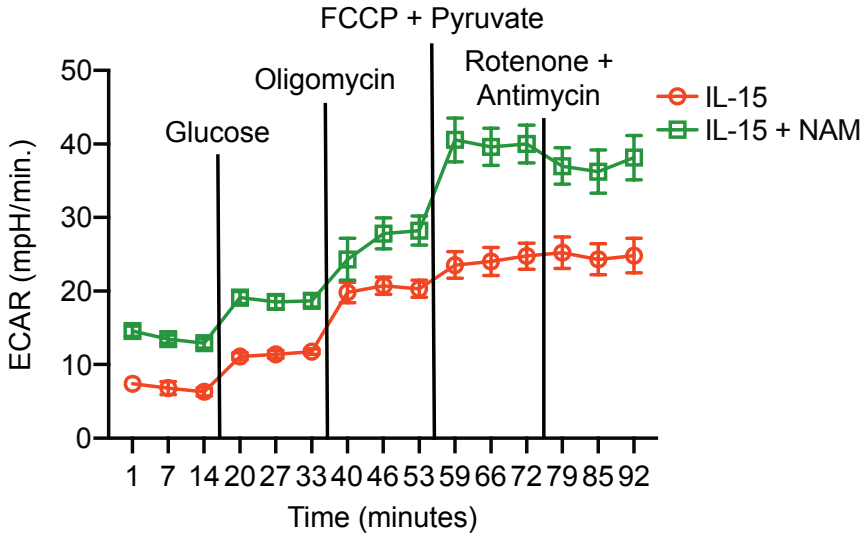
NAM promotes PB NK cell metabolic fitness during ex vivo expansion

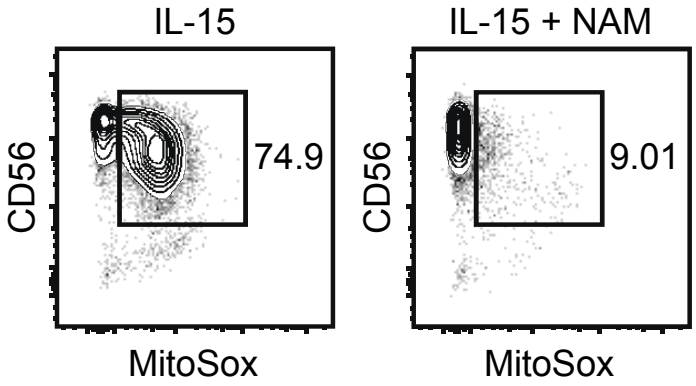




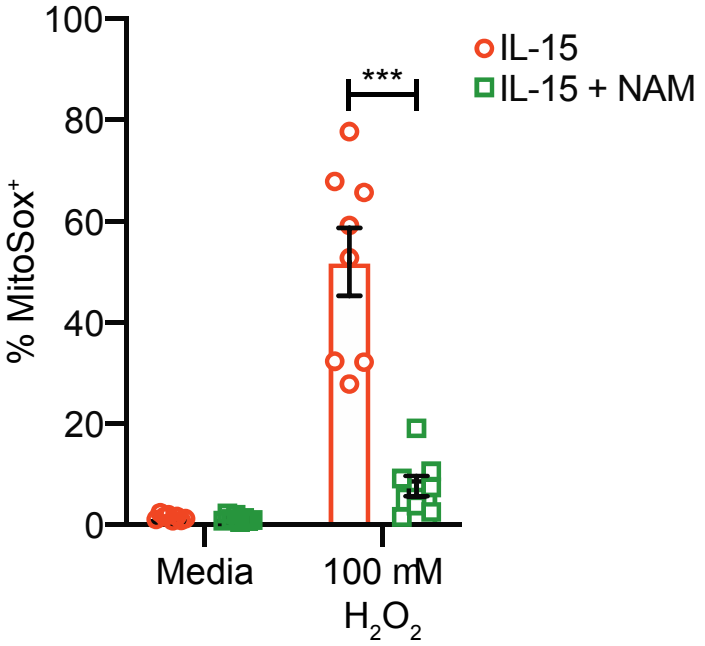
Seahorse assay measures mitochondrial bioenergetics
OCR oxygen consumption rate
ECAR extracellular acidification rate estimates cellular glycolysis

Courtesy F.Chickocki

NAM Protects NK cells against Oxidative stress

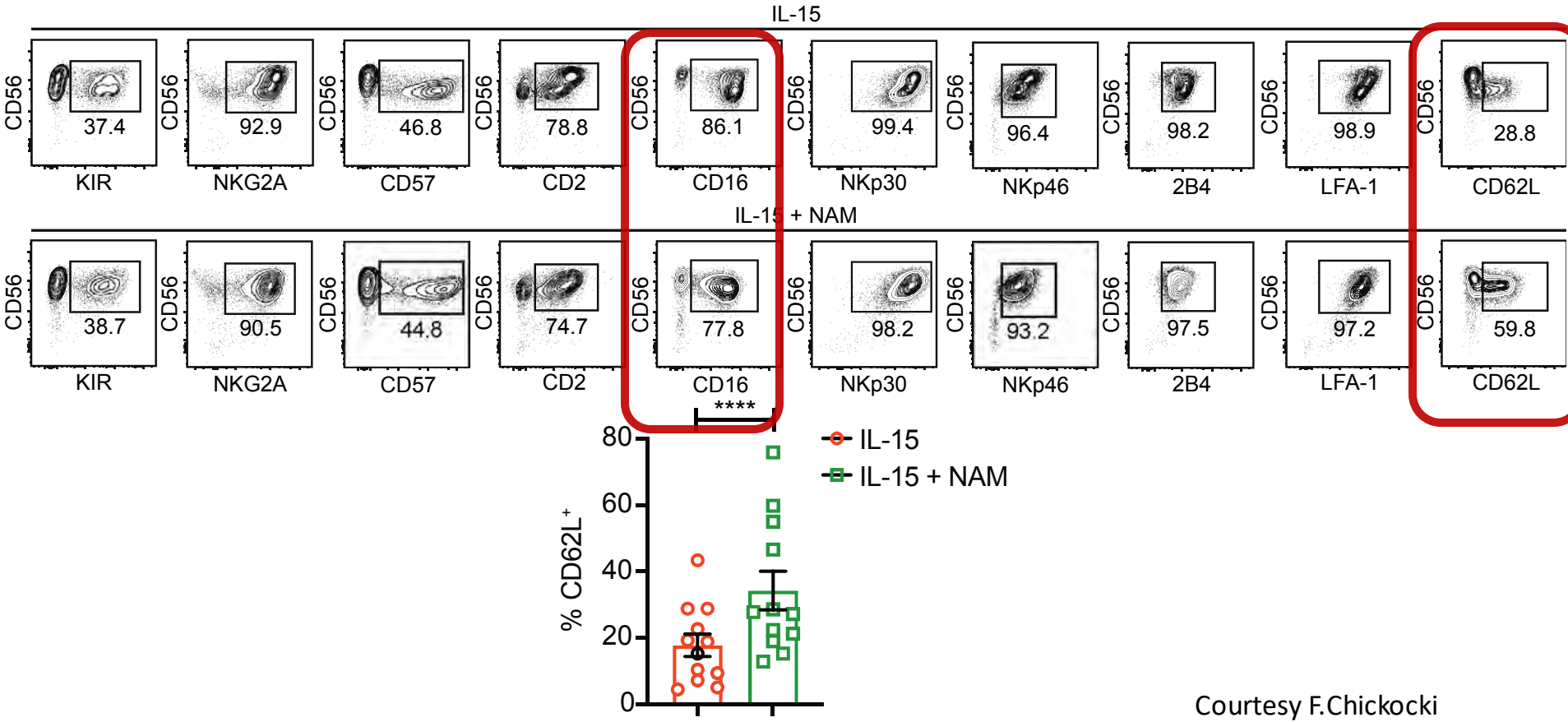


mitochondrial superoxide formation assay after co-culture with hydrogen peroxide



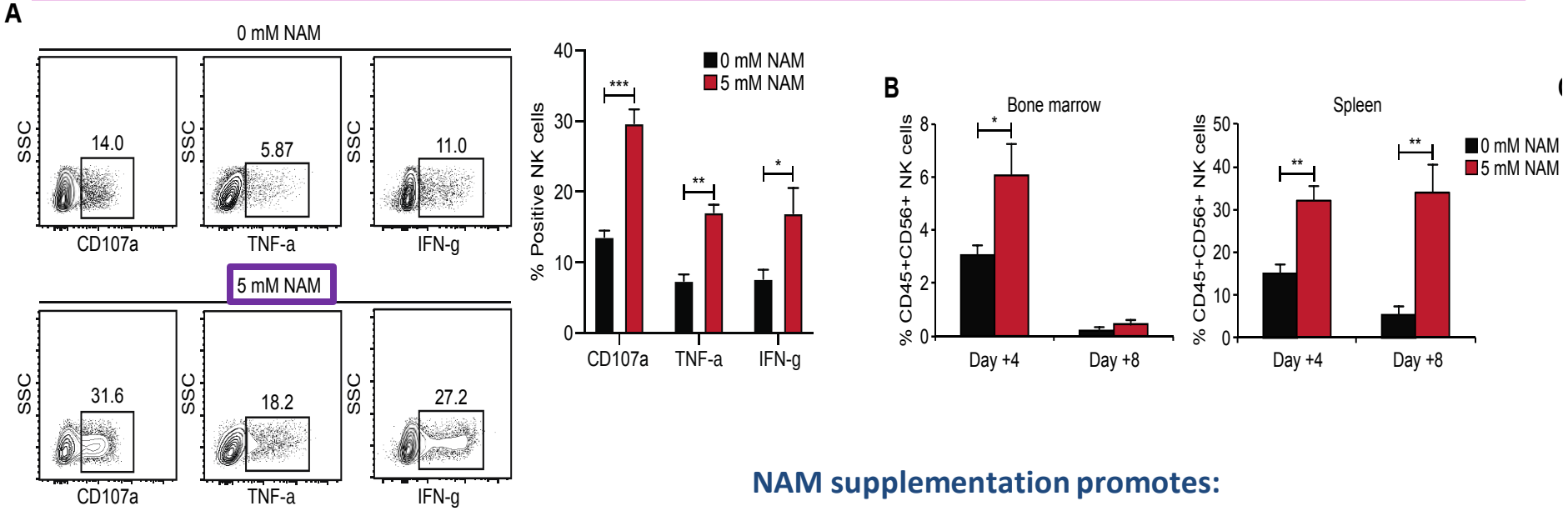
Courtesy F.Chickocki

Selective upregulation of CD62L during NK cell expansion with NAM



Courtesy F.Chickocki

NAM Enhances NK Anti-Tumor Function and Trafficking to Tissues in Animal Models



NAM supplementation promotes:

- NK cell killing and cytokine production
- Tissue trafficking in animal model
- Antibody mediated killing

Peled, T., Brachya, G., et al: Blood 2017, 130:657.

Phase 1 Trial of GDA-201 NAM-Expanded Allo NK Cells (GDA-201) in Patients with Refractory NHL and Multiple Myeloma (MM)

Objective: To evaluate outcomes of GDA-201 in combination with rituximab in patients with R/R NHL

Patients:

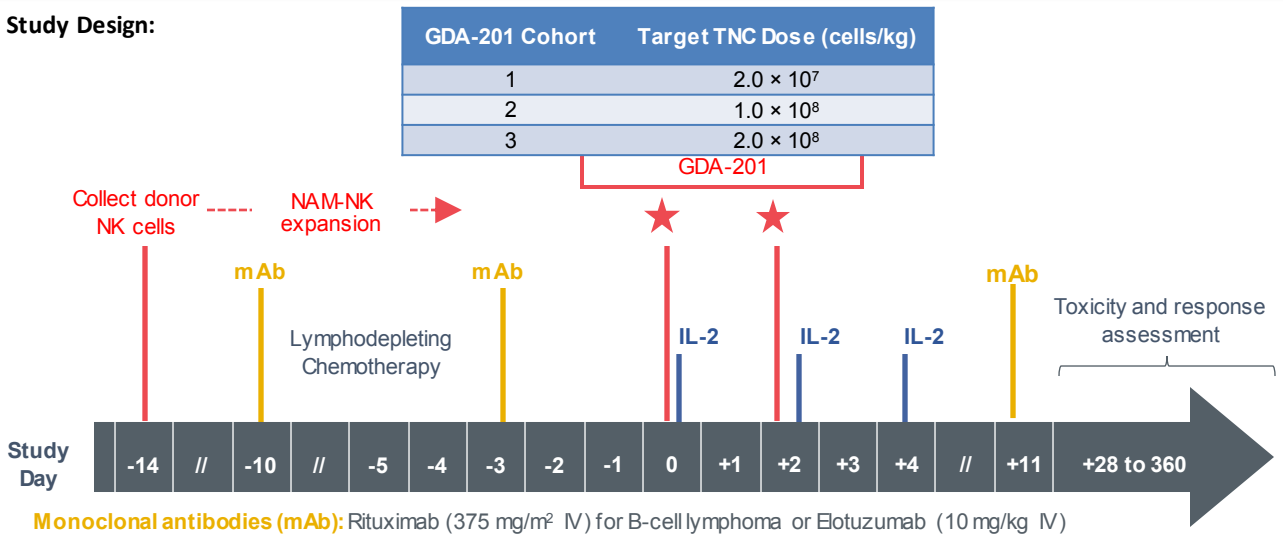
- ≥18 years of age with CD20-positive* B-cell R/R NHL that has failed conventional therapy
- Measurable disease >1.5 cm
- HLA–haploidentical or mismatched related donor
- Karnofsky Performance Scale score ≥60%

Endpoints:

- Safety, dose-limiting toxicities
- ORR, CR, PR, DoR, PFS, OS

Study Design:

GDA-201 Cohort	Target TNC Dose (cells/kg)
1	2.0×10^7
2	1.0×10^8
3	2.0×10^8



Monoclonal antibodies (mAb): Rituximab (375 mg/m² IV) for B-cell lymphoma or Elotuzumab (10 mg/kg IV) for multiple myeloma

Lymphodepleting chemotherapy: Cyclophosphamide (400 mg/m² IV × 3d) and fludarabine (30 mg/m²/d IV × 3d)

IL-2: 6 million units SC

*Confirmed by flow cytometry or immunohistochemistry. CR, complete response; DoR, duration of response; HLA, human leukocyte antigen; IL, interleukin; IV: intravenous; mAb, monoclonal antibody; NAM, nicotinamide; NHL, non-Hodgkin lymphoma; NK, natural killer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; SC, subcutaneous; R/R, relapsed or refractory; TNC, total nucleated cell.

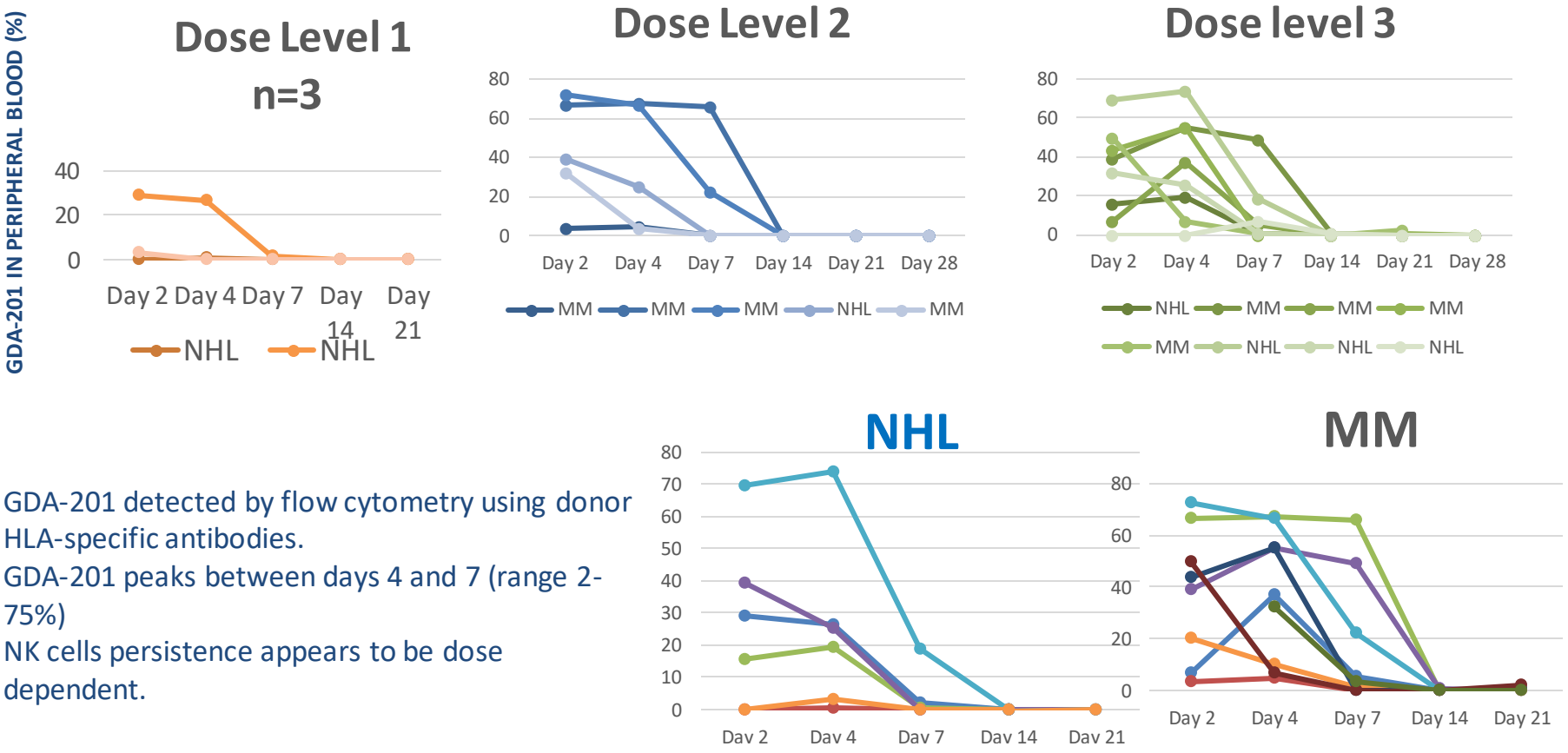
Phase 1 GDA-201 Study: Patients Characteristics

Patient Demographics	Total (N=36)	NHL (n=20)
Age, median (range), years	61 (46–83)	**60 (46–83)**
Sex: male/female, n	21/14	**11/9**
Multiple myeloma, n NHL, n	16 20	**–** **20**
Diffuse large B-cell lymphoma	–	**9**
Follicular lymphoma	–	**10**
Mantle cell lymphoma	–	**1**
Disease status, n (%)		
Relapsed	28 (80)	**17 (85)**
Refractory	7 (20)	**3 (11)**
Stage III–IV (NHL only), n (%)	–	**16 (80)**

Patient Demographics	Total (N=36)	NHL (n=20)
Number of lines of therapies, median (range)	4.5 (1–10)	**3 (1–8)**
Prior autologous transplant, n (%)	16 (47)	**3 (17)**
Prior allogeneic transplant, n (%)	1 (3)	**1 (5.6)**
KPS 80 or less, n (%)	16 (47)	**8 (45)**
GDA-201 cell dose, median in 10^7/kg (range)	**14.3 (2.0–26.0)**	**10.2 (2.0–26.0)**

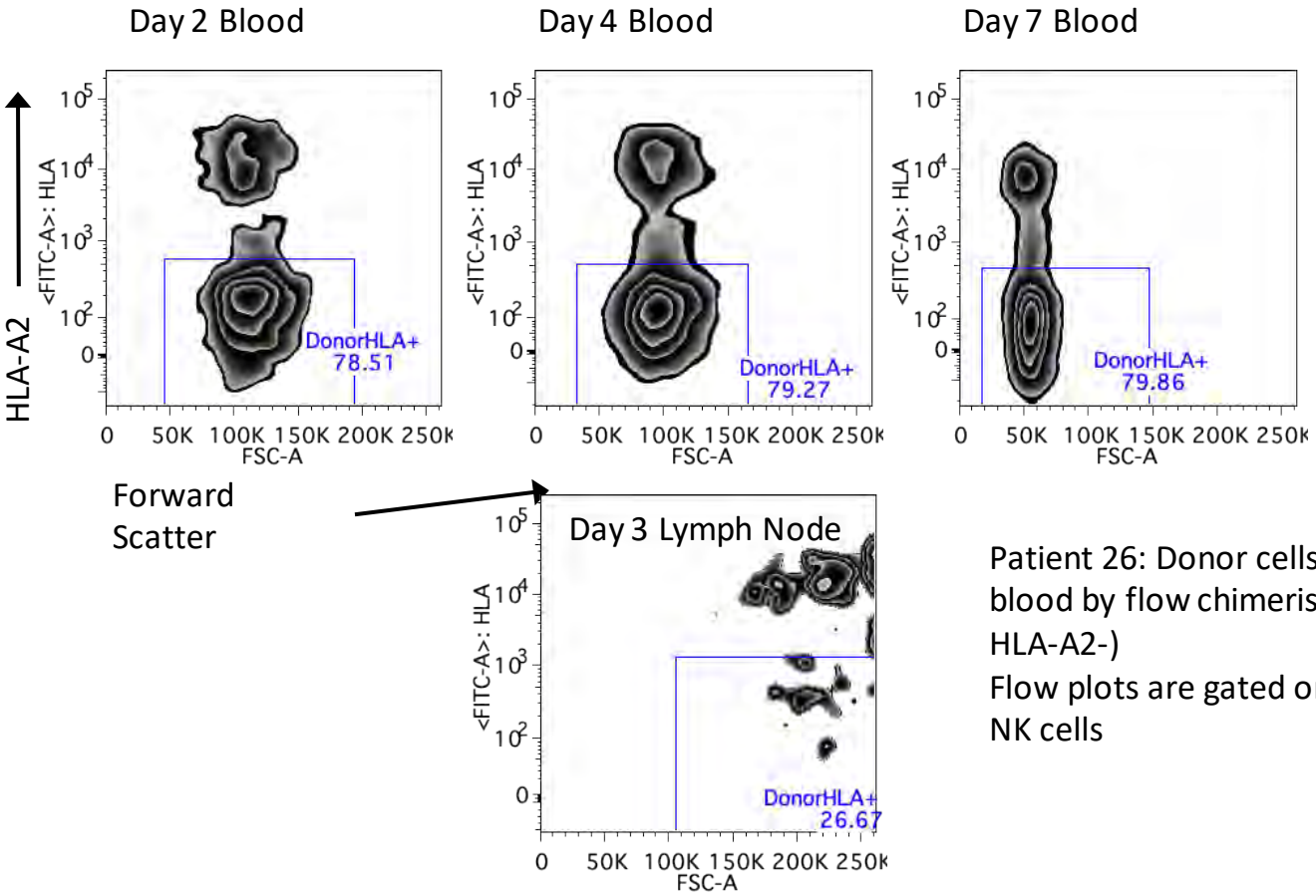
Bachanova, ASH 2021

Persistence and Expansion of GDA-201 (as % of all NK cells)



- GDA-201 detected by flow cytometry using donor HLA-specific antibodies.
- GDA-201 peaks between days 4 and 7 (range 2-75%)
- NK cells persistence appears to be dose dependent.

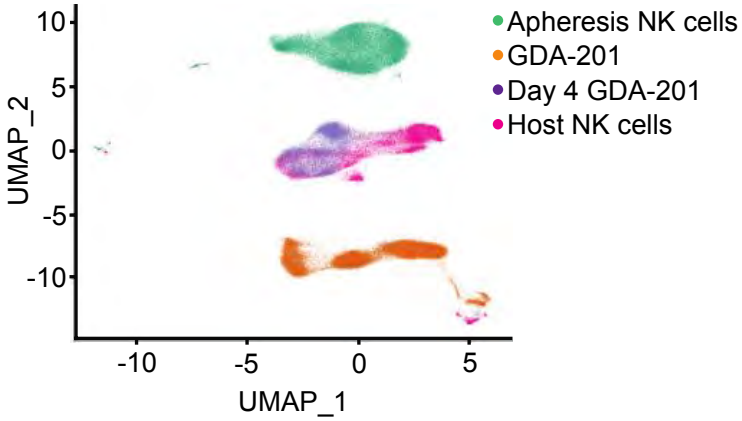
GDA-201 Cells Traffic to Marrow and Lymph Nodes

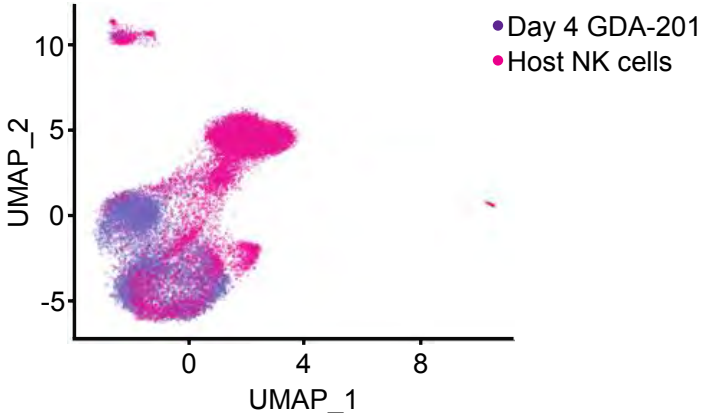


Patient 26: Donor cells detected in blood by flow chimerism (donor is HLA-A2-)

Flow plots are gated on CD56+CD3- NK cells

Metabolic Re-programming with NAM Alters scRNA-seq Expression

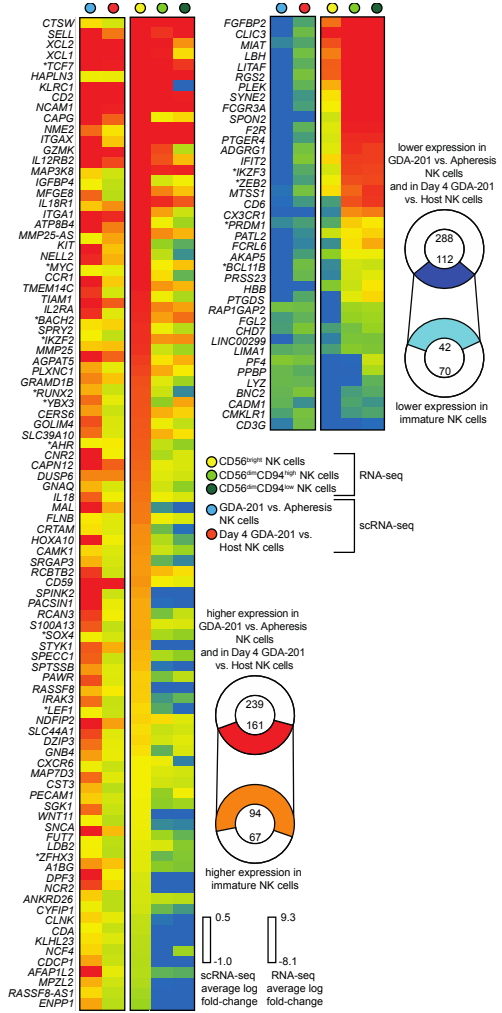




- Distinct differences in gene expression are observed in GDA-201 vs donor aphered NK cells
- Day 4 GDA-201 and Host NK cells cluster more closely, likely reflecting adaptation of the product in the host.

Frank Cichocki, Justin Hwang, unpublished

GDA-201 cells resembles more immature stages of NK cell development



- NAM induced durable changes in gene expression of donor NK cells
- Differential gene expression of GDA-201 cells resembles more immature stages of NK cell development (NK565^{bright}).
- Many genes associated with maturity were downregulated

Frank Cichocki, PhD
Justin Hwang, PhD
unpublished

GDA-201 trial: Safety & Efficacy

Safety

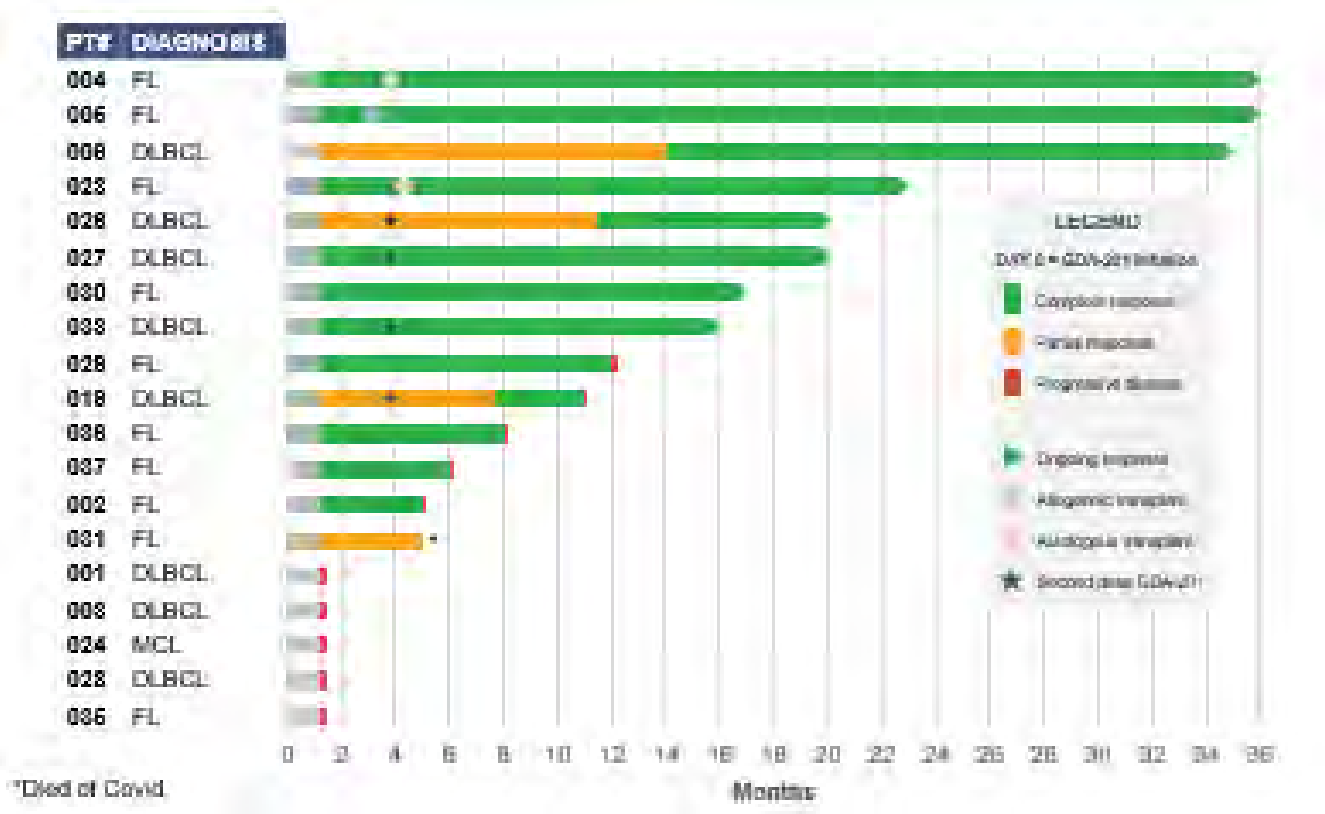
- The most common grade 3/4 TEAEs were:
  - Thrombocytopenia (n=9)
  - Hypertension (n=9)
  - Neutropenia (n=4)
  - Febrile neutropenia (n=4)
  - Anemia (n=3)
- Adverse events of special interest (cytokine release syndrome, neurotoxic events, graft-versus-host disease, or bone marrow aplasia) were not observed
- One patient died of *Escherichia coli* sepsis

Efficacy in Non-Hodgkin lymphoma (19 evaluable patients)

- **ORR was 74%**
- **CR rate 65% (n=13; 5 with DLBCL and 8 with FL)**
- **1 patient had PR**
- 4 NHL patients underwent re-treatment with GDA-201; 2 patients had further deepening of response from PR to CR

Bachanova, ASH 2021

Duration of response after GDA-201



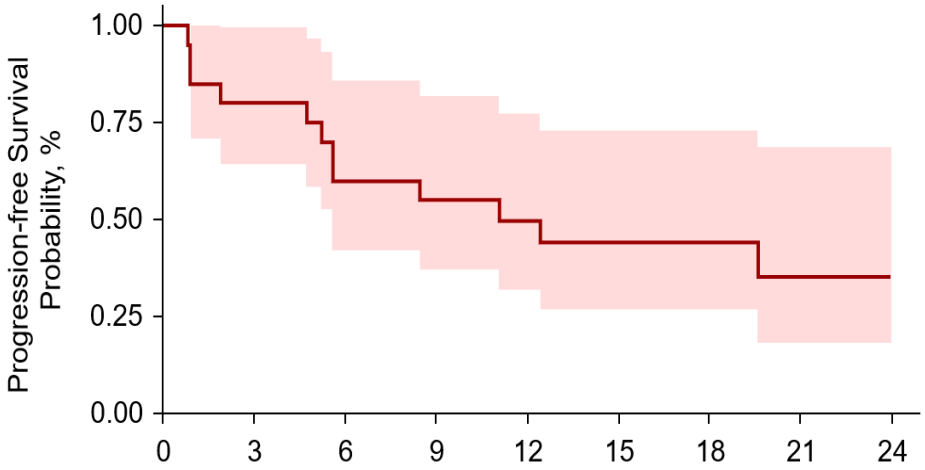
Median duration of response was 16 months (range 5-36 months)

Bachanova, ASH presentation 2021

Survival at a median follow-up of 11 months (range, 1–36)

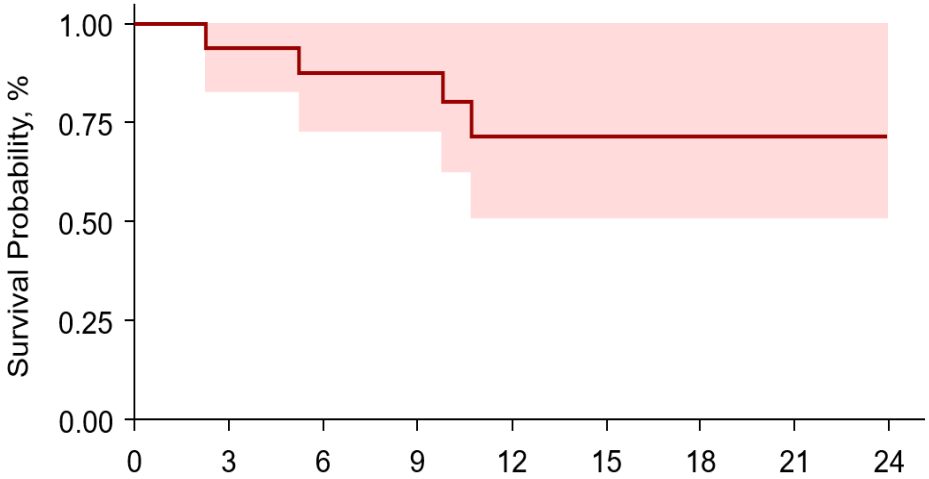
Progression-free Survival

- 1- year PFS were 50% (95% CI, 27%–69%)
- 2-year PFS was 35% (95% CI, 14%–58%),



Overall Survival

- 2-year OS was 78% (95% CI, 51%–91%)

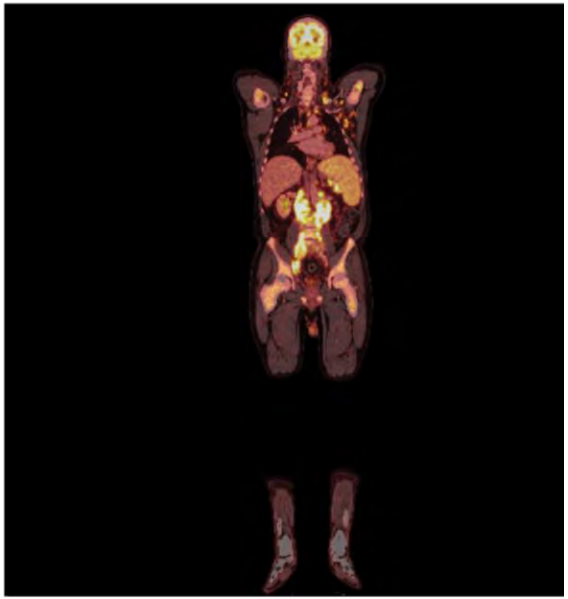


Bachanova, ASH 2021

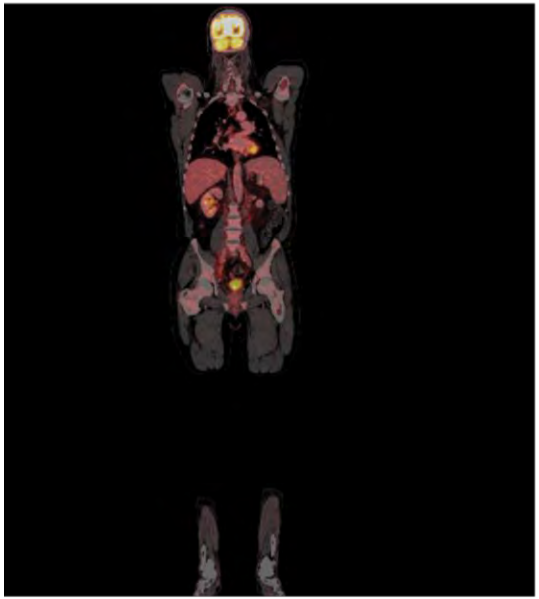
Patient 004

- 60-year-old man with FL stage IVB
- Prior therapy: BR
- Relapse R-CHOP, and R-ICE – no response
- Day 28 post GDA-201: Complete response

Baseline



1 month post GDA-201

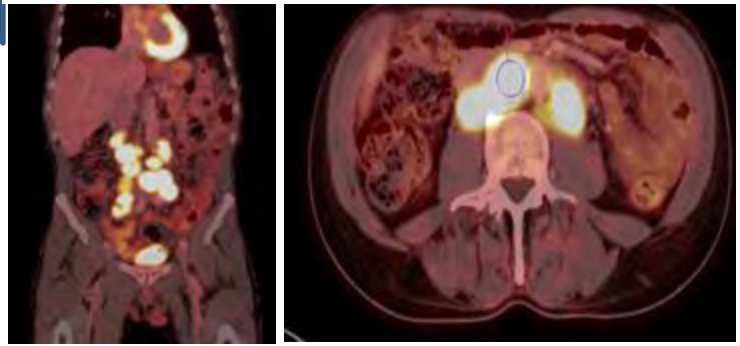


Patient 004: Radiographic Complete Response

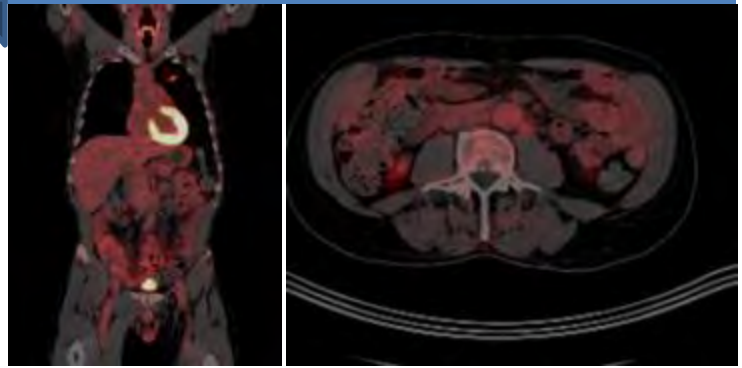
Patient 009

- 57-year-old man with history of CLL and Richter's transformation - large cell lymphoma, measurable retroperitoneal lymph nodes at baseline
- Prior therapy: FCR-light, Rituximab/Bendamustine Ibrutinib/Revlimid, R-CHOP, Venetoclax/Rituximab
- Allogeneic matched sibling HSCT
- Relapse at 6 months
- Treated with GDA-201
- 28 day response- Partial Response
- 6 months: PR with continued tumor shrinkage
- 12 months: Complete response

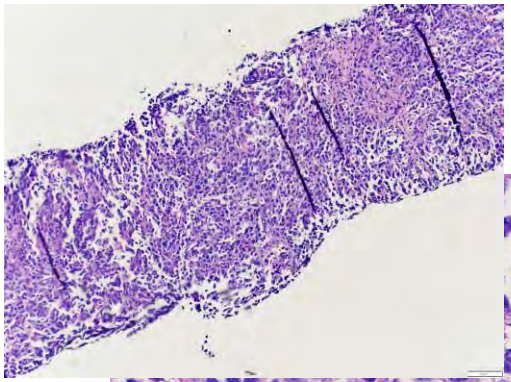
Pt 009: Baseline



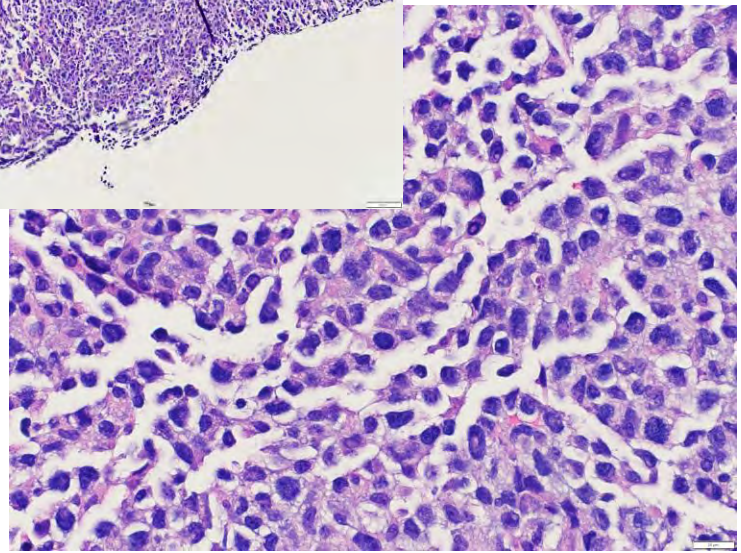
Pt 009: 6 month post GDA-201

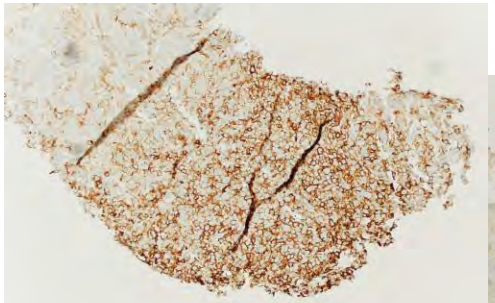


Pre-treatment tumor biopsy patient 009



B-cell lymphoma



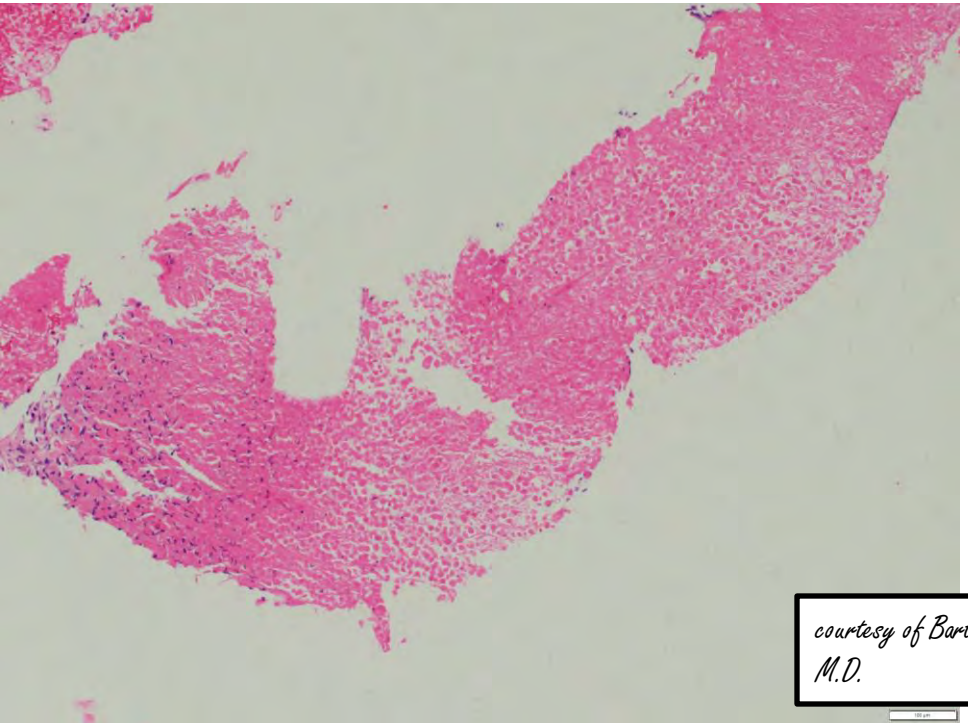


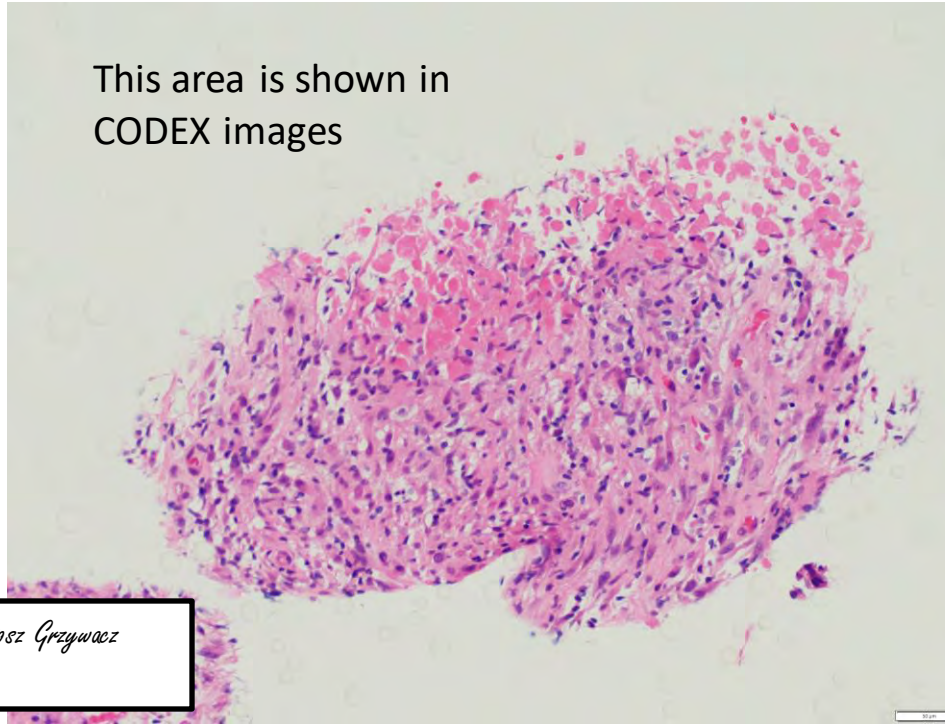
CD20 stains lymphoma B cells

CD3

courtesy of Bartosz Grzywacz MD

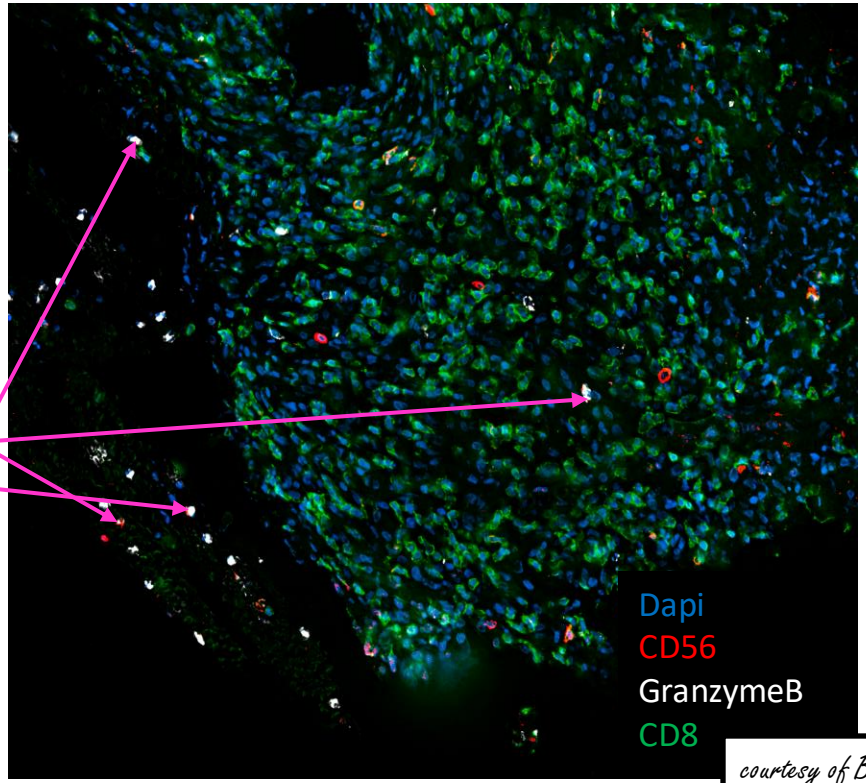
Post – treatment day 16 biopsy: necrotic lymphoma cells with ensuing tissue organization and fibrosis with inflammatory infiltrates.





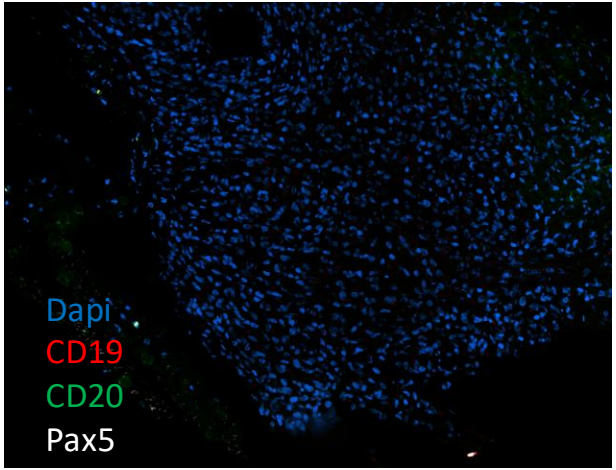
courtesy of Bartosz Grzywacz M.D.

Detection of NK cells in tissues by CODEX at after GDA-201



CD56+ ,
Granzyme
B+ **NK cells**

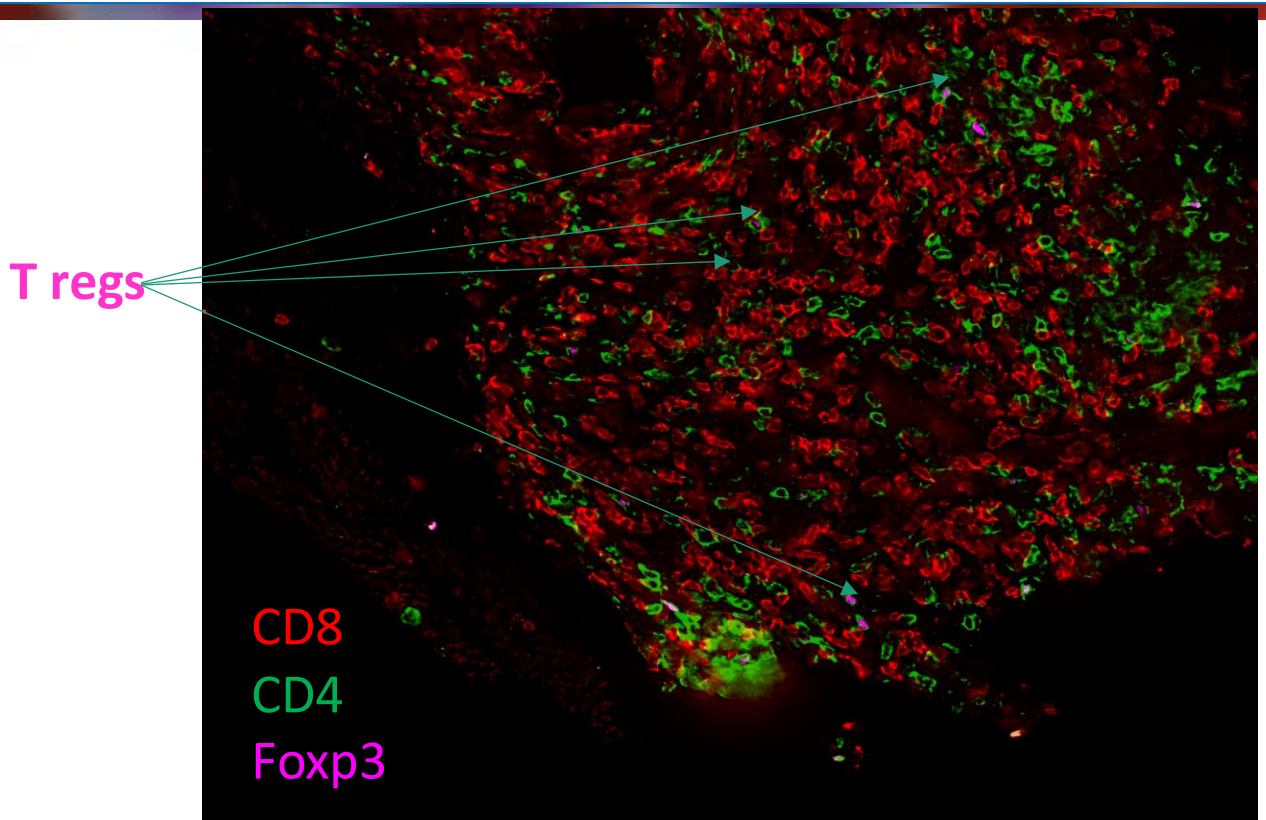
courtesy of Bartosz Grzywacz M.D.



B cell markers: CD19, CD20, PAX5 are <u>all negative</u>

<u>C/w Tumor eradication</u>

T cell infiltrate predominates in post-treatment tumor biopsy



courtesy of Bartosz Grzywacz M.D.

Phase 1 GDA-201 Study Findings and Limits:

- We developed a novel cell product manufactured with nicotinamide and void of genetic engineering
- Future directions include cryopreservation, HLA mismatching and exploration of multiple treatment cycles.
- **Multi-center Phase 1/2 study started enrollment in May 2022**

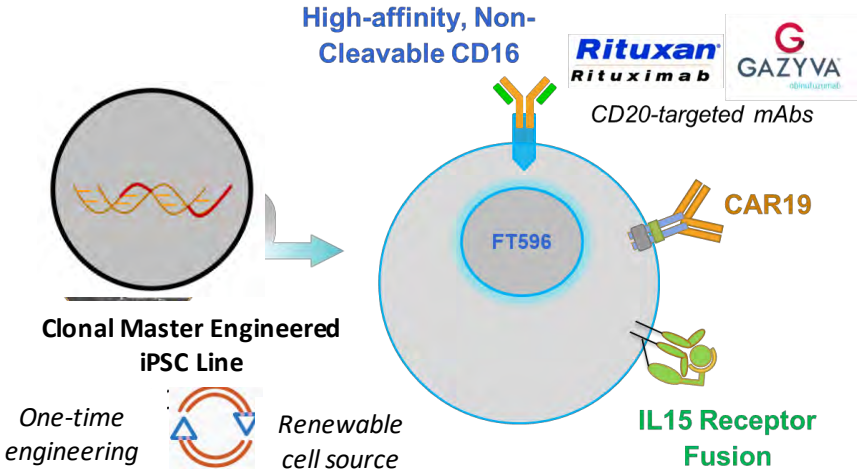
Clinical Challenges in The Field

Cell persistence
Tumor specificity
Optimal expansion and trafficking
Cross-talk with adaptive immune system
Tumor escape in suppressive immune milieu

Off-the-Shelf, Multi-Targeted CAR iNK Cells for Cancer Therapy

Engineered from iPSC Line and Triple Genetically Modified

Cell source	Advantages	Disadvantages
iPSC	-High proliferative capacity -Homogeneous product	-Immature phenotype -Low ADCC due to low CD16 expression - Long culture condition



hnCD16: High-affinity 158V, non-cleavable CD16 Fc receptor to augment ADCC, enabling dual antigen targeting

CAR19: targets B-cell antigen CD19, optimized for NK cell biology, contains a NKG2D transmembrane domain, a 2B4 co-stimulatory domain and a CD3-zeta signaling domain

IL-15RF: Interleukin-15 receptor fusion, potent cytokine complex to promote survival and proliferation of NK cell and CD8 T cells

Jode P Goodridge et al, ASH Abstract 2019;
FATE Therapeutics with permission

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AMERICAN SOCIETY OF HEMATOLOGY

Jeff Miller, MD
Frank Cichocki, PhD
Martin Felice,PhD
Daniel Weisdorf, MD

RNAseq
Justin Hwang, PhD

Pathology/CODEX
Bartosz Grzywacz, MD

GMP Cell Processing Facility
David McKenna, MD
David Sumstad
Dianne Kadidlo

Translational Therapy Core Lab
Rose Wangen

Cell Therapy Clinical Group

BMT physicians
Joe Maakaron,MD
Claire Career, NP
Tasha Kell, PA

CTO/CGI:
Ashley Lyle, RN
Dixie Lewis, RN
Emily Steen, RN
Roby Nicklow, RN
Jaime Wurth regulatory/IND
Jill Aughey IND, protocol

BMT/CT Program
Tammy Grainger, NC, MS, RN
Carol Rose, RN
Pharmacy BMT group