

# Tumour arginine addiction subverts the anti-cancer immune response

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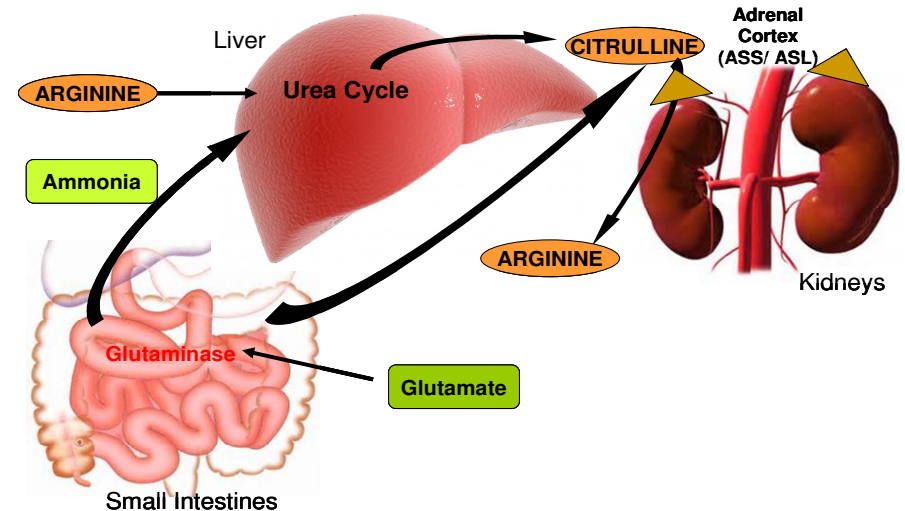
# Overview

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- Acute Myeloid Leukaemia (AML)
  - The second most common leukaemia of childhood and increasingly common with older age
- Neuroblastoma
  - The most common extra-cranial solid tumour of childhood
- Common themes
  - Embryonic cells with developmental arrest and failure to mature, in combination with malignant proliferation
  - Immune alterations
  - Cytopenias at diagnosis (not due to bone marrow effacement)
  - Poor prognosis

# Whole body Arginine Homeostasis

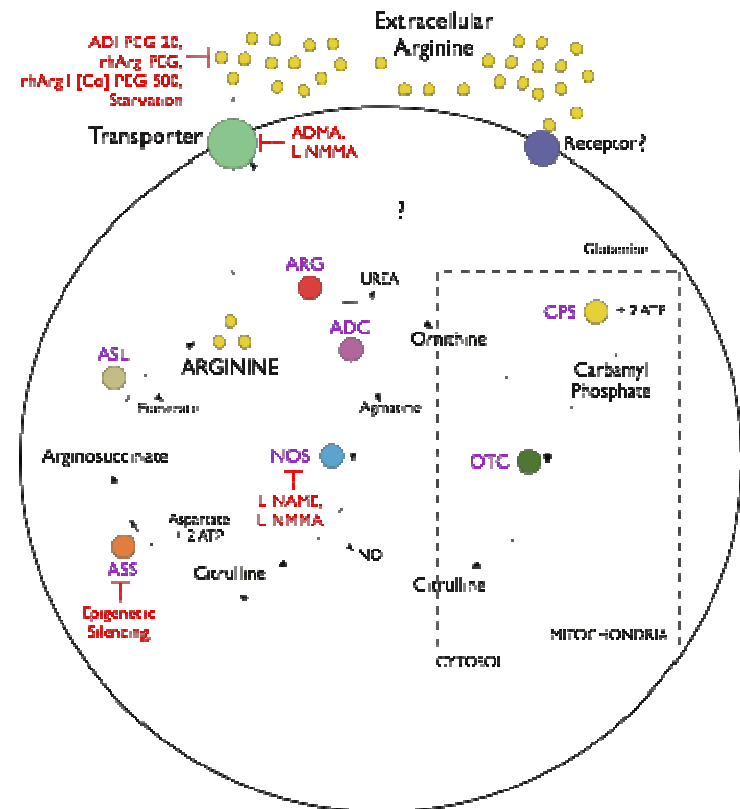
- L-arginine is an amino acid found in virtually all peptides, enzymes and proteins.
- Key metabolic pathways and cellular events
  - Urea Cycle
  - Nitric Oxide formation
  - Creatine and polyamine production
  - Cell cycle signaling
  - Protein synthesis



- Consumed in the diet (85%)
- Arginine can also be synthesised (5-15%) (simple view):
  - Glutamine converted to citrulline and ammonia in the enterocytes
  - (Ammonia enters the urea cycle in the liver)
  - Citrulline passes through the liver and converted to arginine in the proximal tubules of kidney via Argininosuccinate Synthetase/ Argininosuccinate Lyase (ASS/ASL) enzyme system. Arginine is released back into the blood
  - Added complexity (liver produced arginine is metabolised by the liver; dietary changes)
- Tight compartmentalization in various organs and tissue spaces and under stringent homeostatic control in blood (NR:70-120 uM).

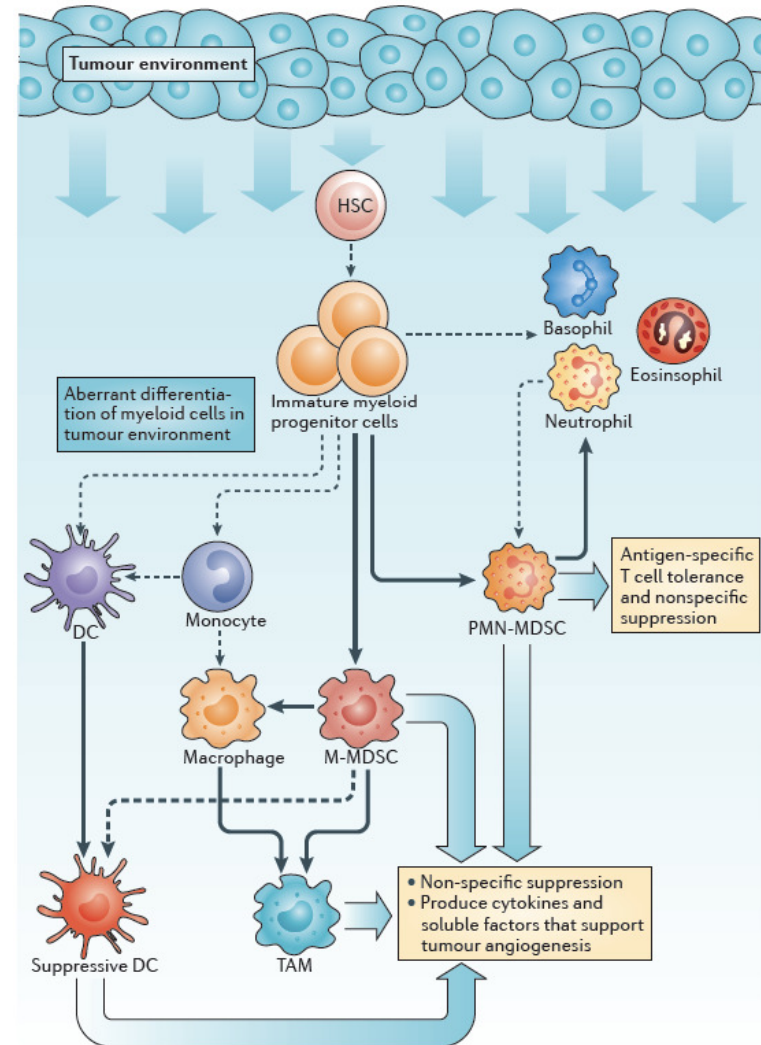
# Intracellular Arginine homeostasis

- Uptake via CAT family of surface proteins
  - ? A mechanism of arginine sensing
- The majority of somatic cells can use citrulline as a surrogate to resynthesise arginine via intracellular ASS/ASL/OTC
- Arginine is considered a semi-essential amino acid
  - under conditions of high demand (pregnancy, inflammation, trauma) whole body arginine can become limiting



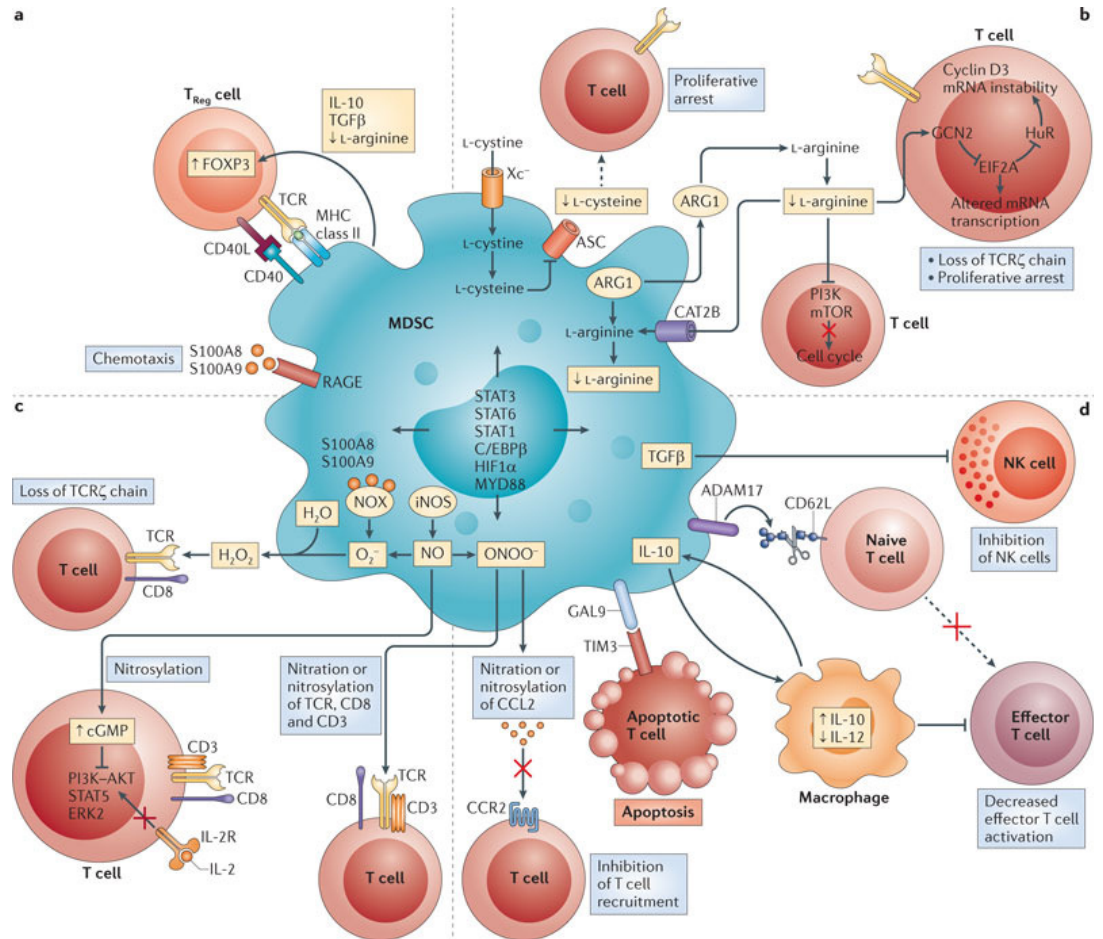
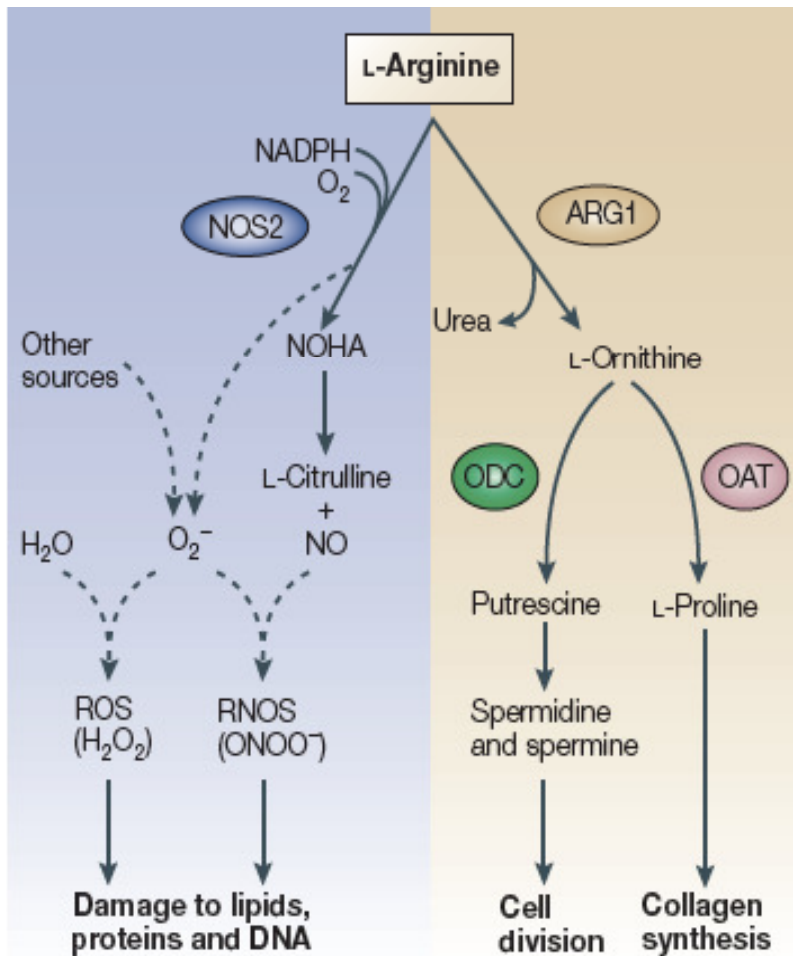
# Myeloid cells and arginine

- Non-malignant myeloid cells metabolise arginine to alter the balance of immunity
  - Tumour-associated Macrophages (TAMs)
  - Myeloid-derived suppressor cells (MDSCs)
- Cancer (Reviewed in Mussai et al. 2011)
- Infection
- Pregnancy



Gabrilovich, Ostrand-Rosenberg, Bronte 2012

# Myeloid-T interactions



# Arginine *addiction* by cancer cells

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- Certain cancers are unable to re-synthesise arginine because of down-regulated, or absent expression of OTC/ ASS/ ASL
- **Malignant cell** is reliant on extracellular arginine – auxotrophism
- In these cases arginine is therefore an essential amino acid

Hepatocellular carcinoma (Cheng 2007)  
Prostate cancer (Hsueh 2012)  
Pancreatic cancer (Glazer 2011)  
Renal cell carcinoma (Yoon 2007)  
Breast cancer (Wang 2014, Qiu 2014)  
Lung/Mesothelioma (Agrawal 2012; Szlosarek 2006)  
Melanomas (Lam 2011; Yoon 2013)  
Glioblastomas (Sippel 2011) (Pavlyk 2015)  
Diffuse Intrinsic Pontine Glioma (Ching, Baylor)  
Osteosarcoma and lymphoma (Wells 2013; Kobayashi 2010)  
Non-Hodgkin's Lymphoma (Zeng 2013; Shu 2014)  
Neuroblastoma (Mussai et al. 2015; Lin et al. 2014)  
Acute Myeloid Leukaemia (Mussai 2015)  
Acute Lymphoblastic Leukaemia (Mussai 2015)  
T-Acute Lymphoblastic Leukaemia (Kwong-Lam 2013)  
Soft tissue sarcoma (Yan 2011, Takaku 1995)

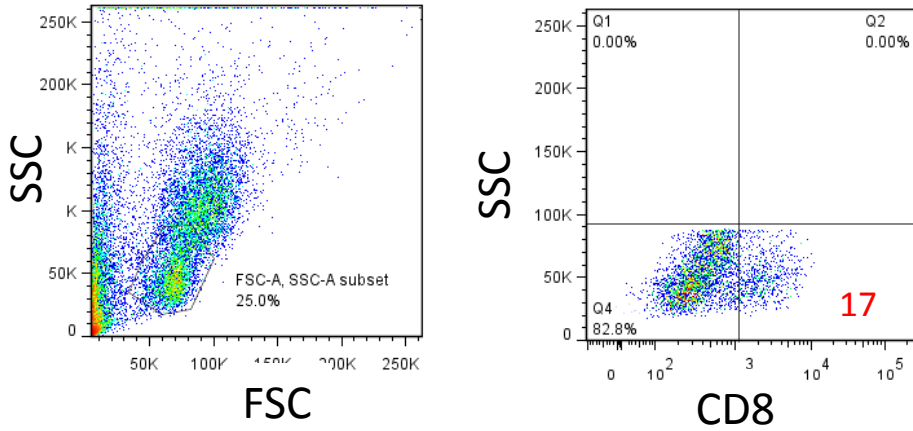
# Observations in AML

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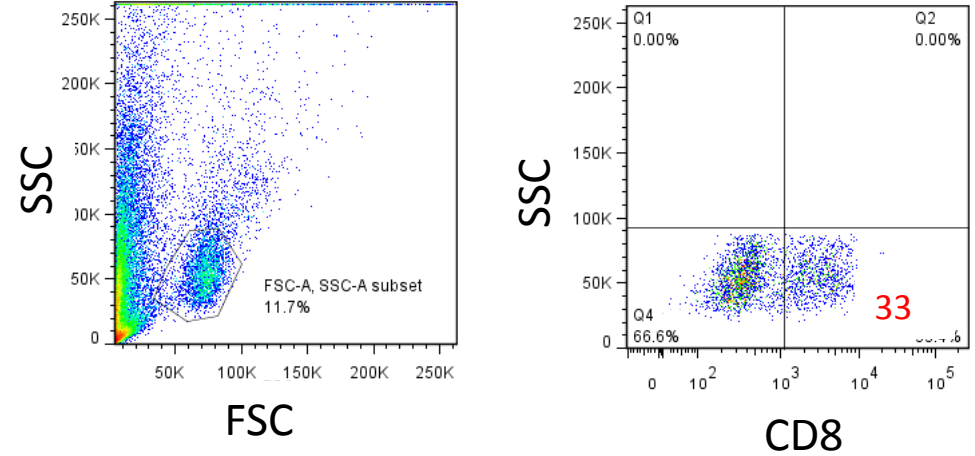


# Impaired anti-AML T cell immunity

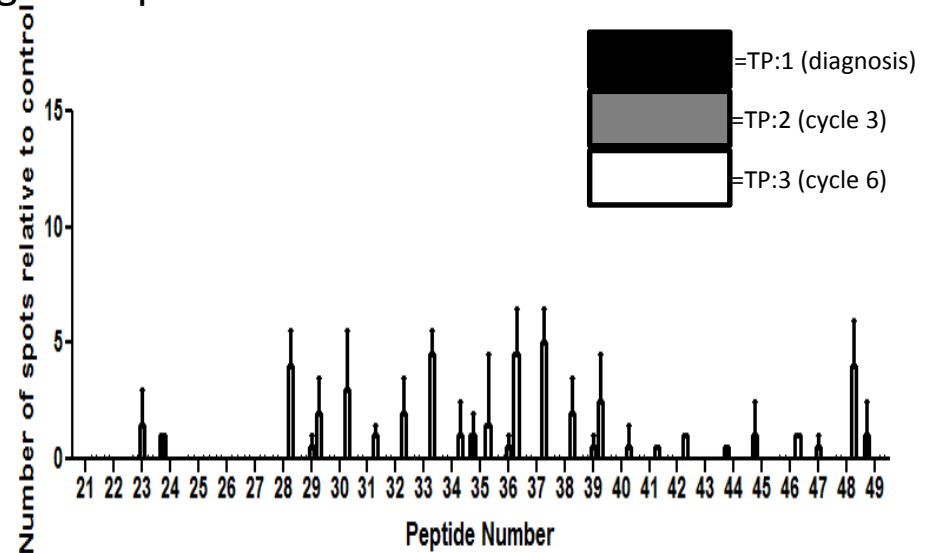
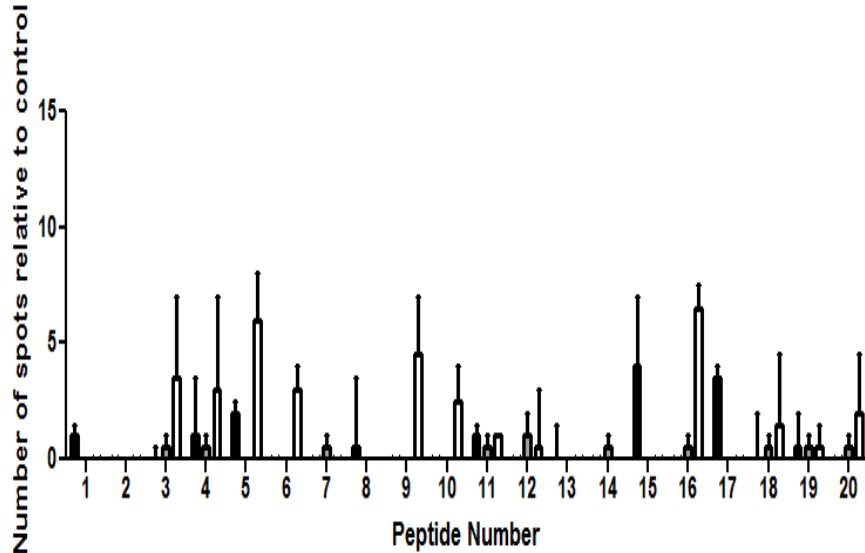
Diagnosis (TP:1)



Post-therapy (TP:3)

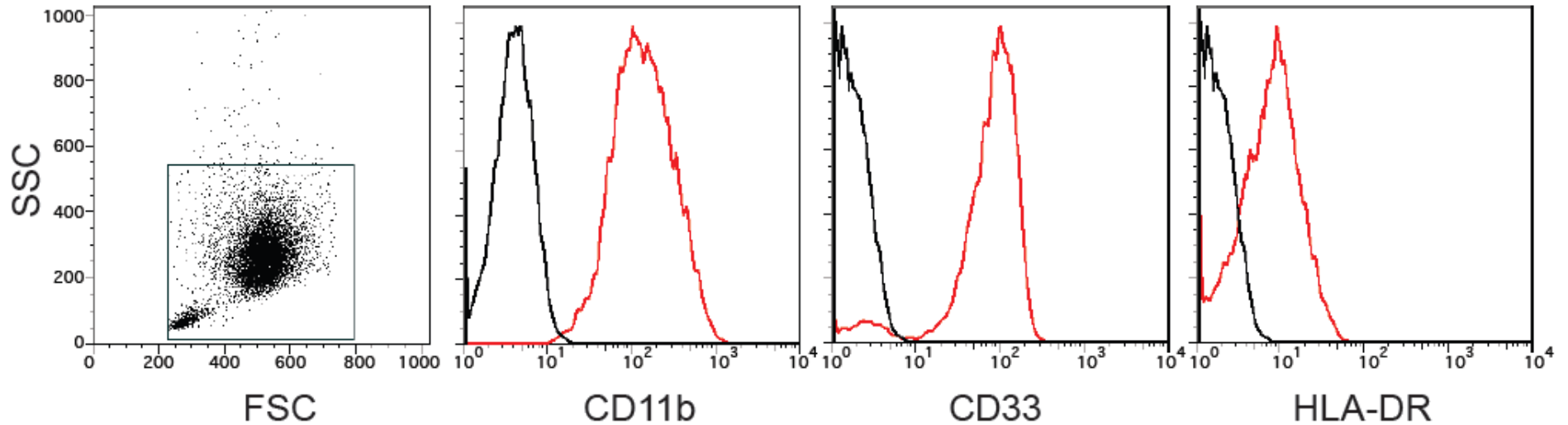


Cancer Testis Antigen responses



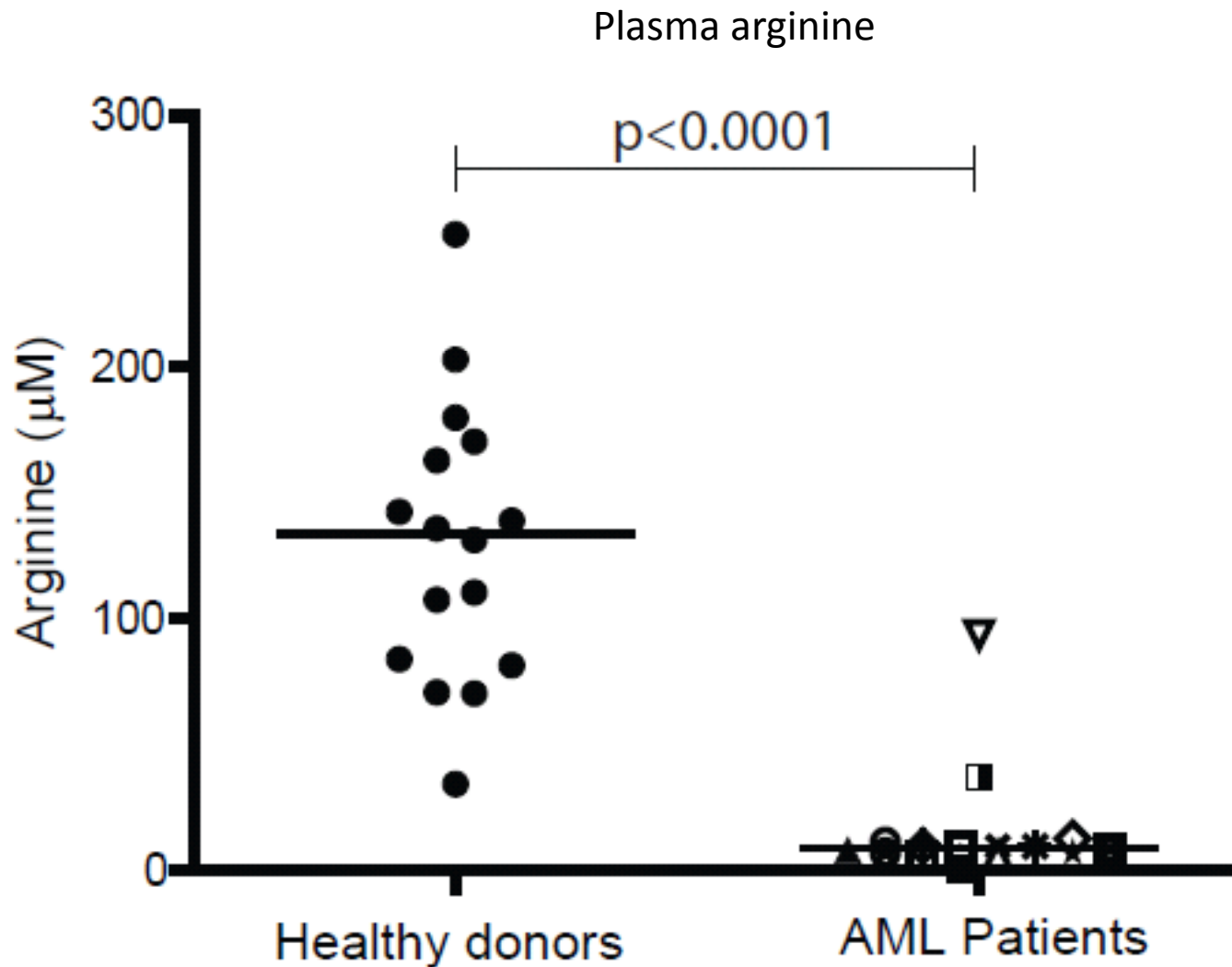
# AML blasts express classical MDSC phenotype

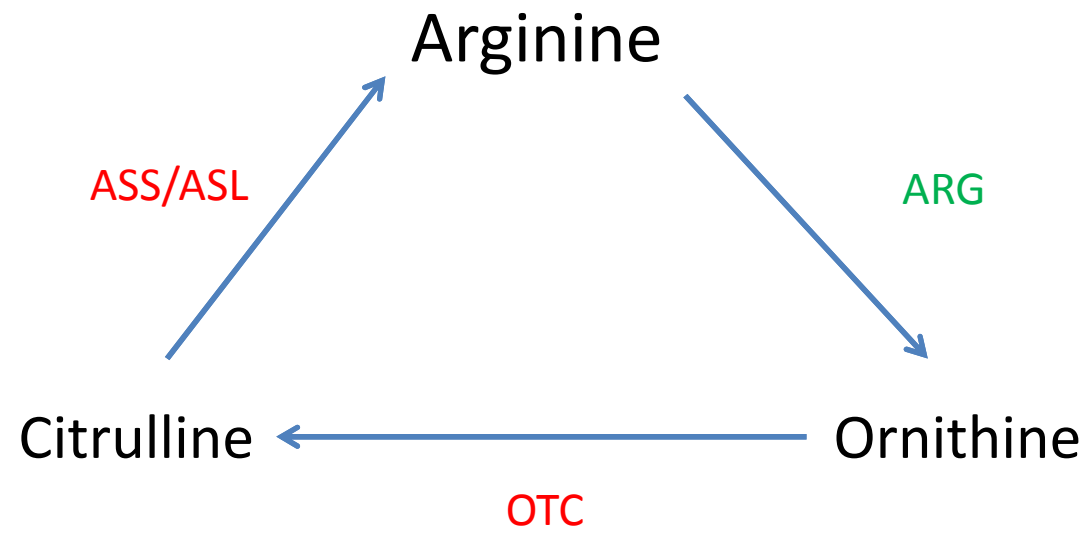
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CD14+ or CD15+ - dependent on maturation stage

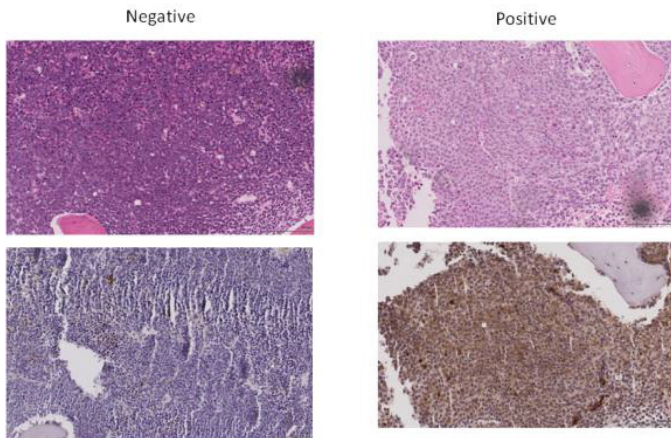
# AML blasts consume extracellular arginine



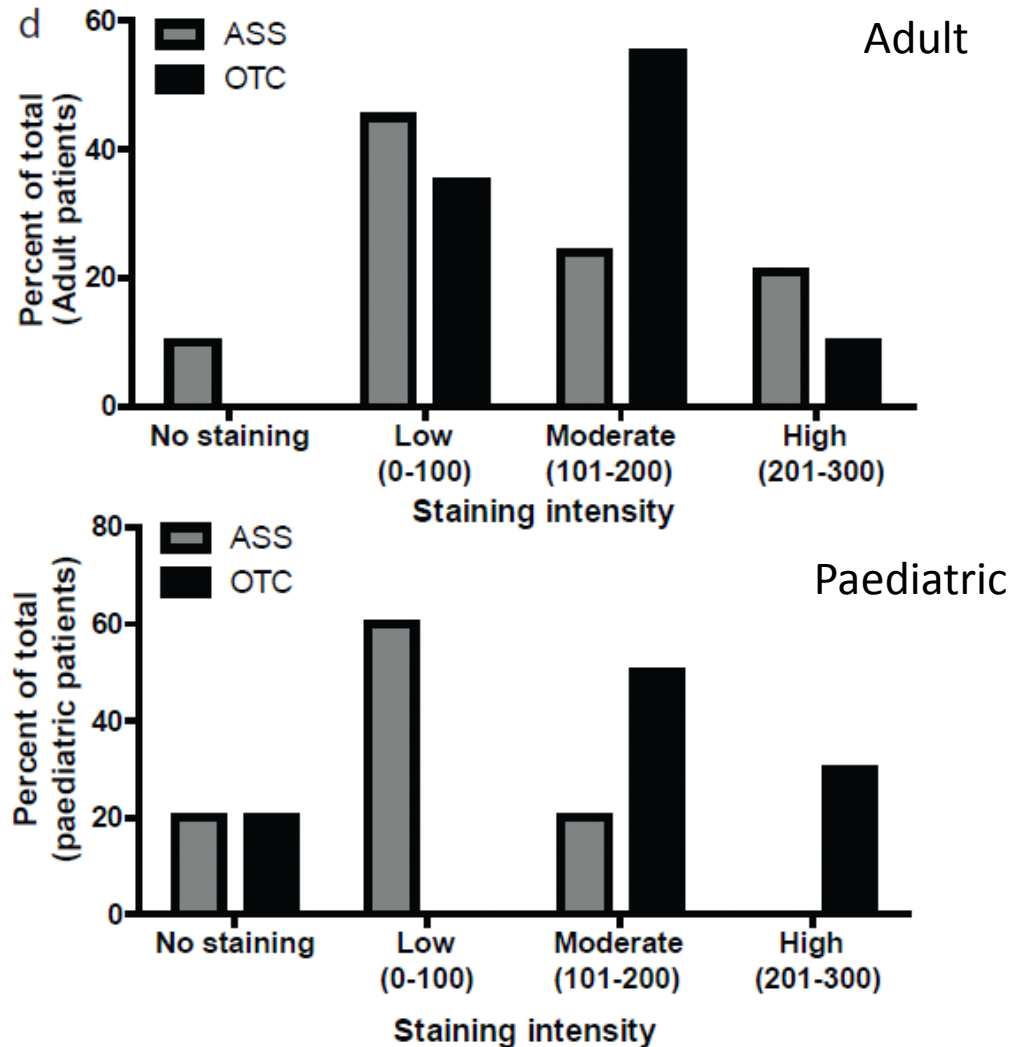
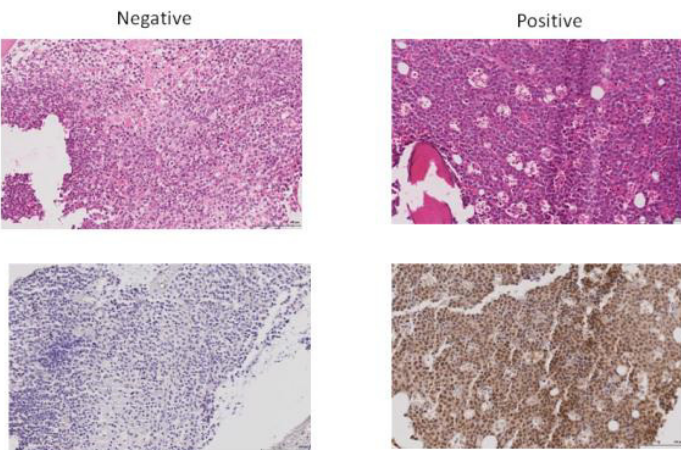


# AML blasts are auxotrophic for arginine

ASS

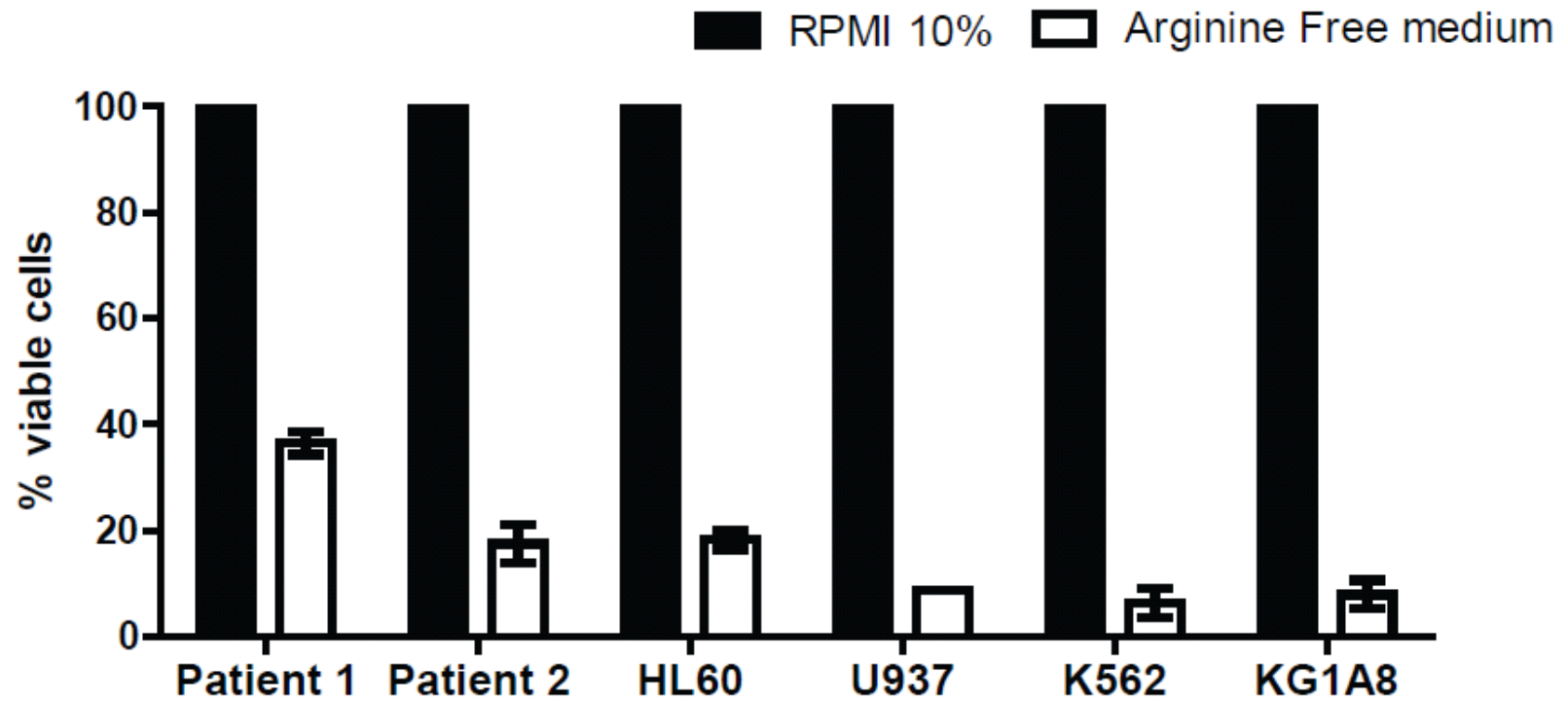


OTC



# Dependence on extracellular arginine for survival

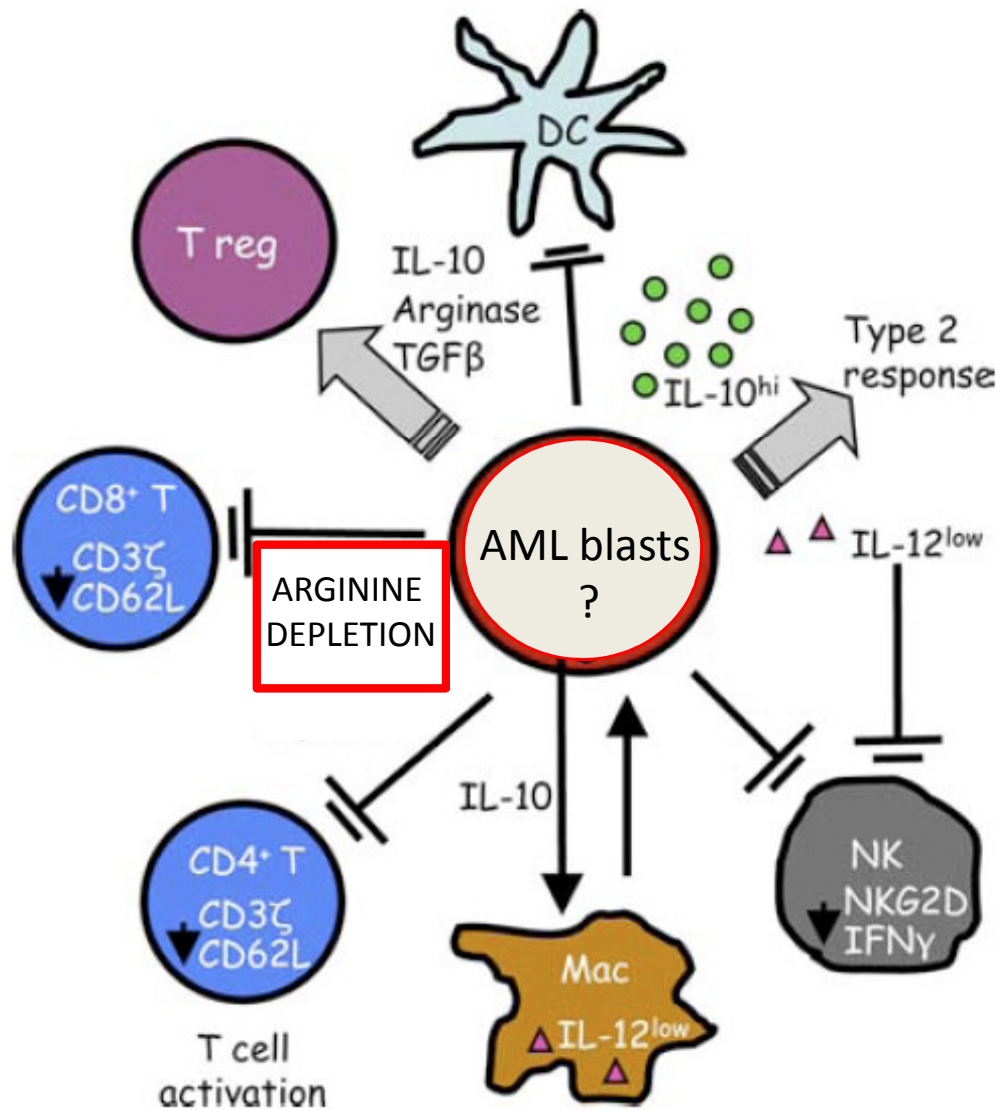
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For more on this story see [Mussai et al. Blood 2015](#)

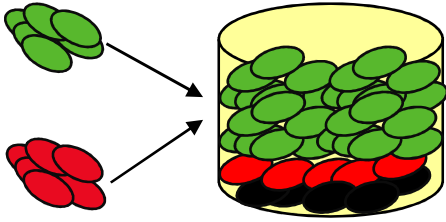
# MDSCs suppress T cell immunity by arginine depletion

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# AML blasts suppress T cell proliferation

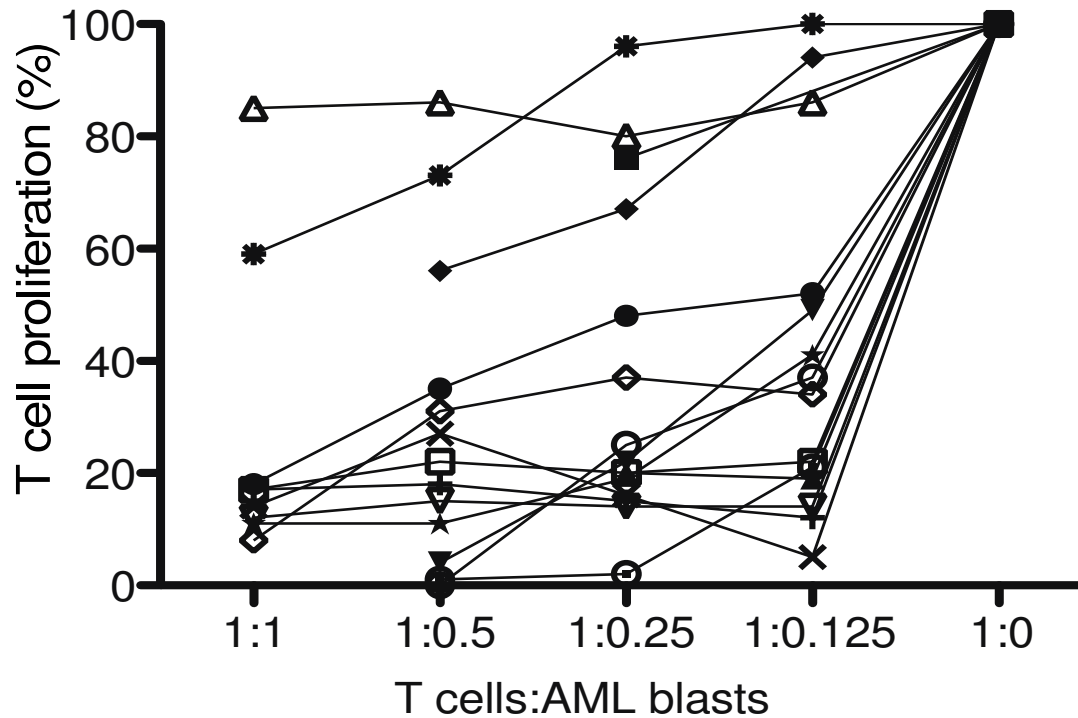
T cells



DC

DC : AML blast : T cells  
 $5 \times 10^4$  :  $1 \times 10^5$  :  $2 \times 10^5$

MLR = Mixed Leukocyte Reaction

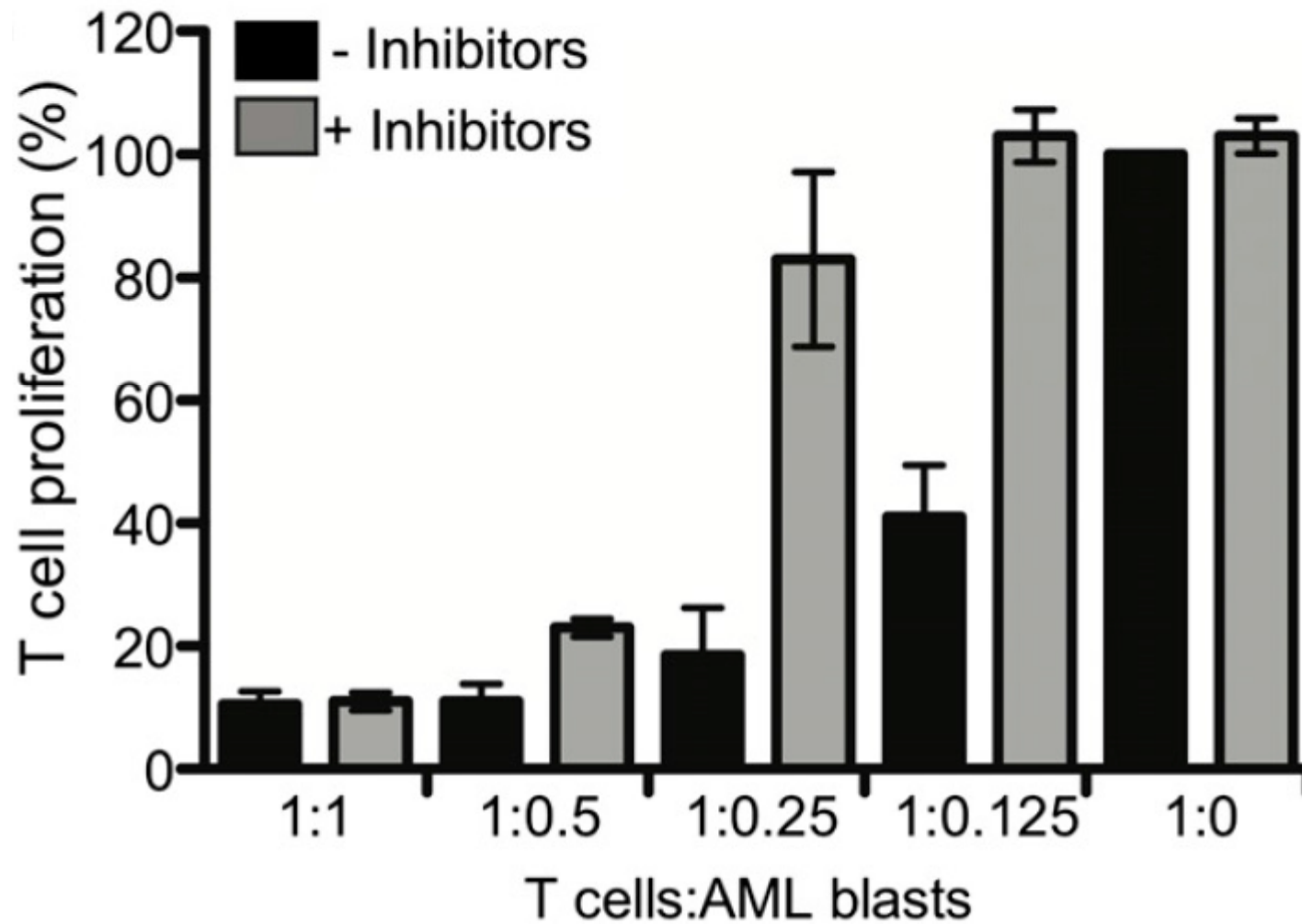


- P1
- P2
- ▲ P3
- ▼ P4
- ◆ P5
- P6
- P7
- △ P8
- ▽ P9
- ◇ P10
- \* P11
- ★ P12
- + P13
- × P14
- ⊖ P15

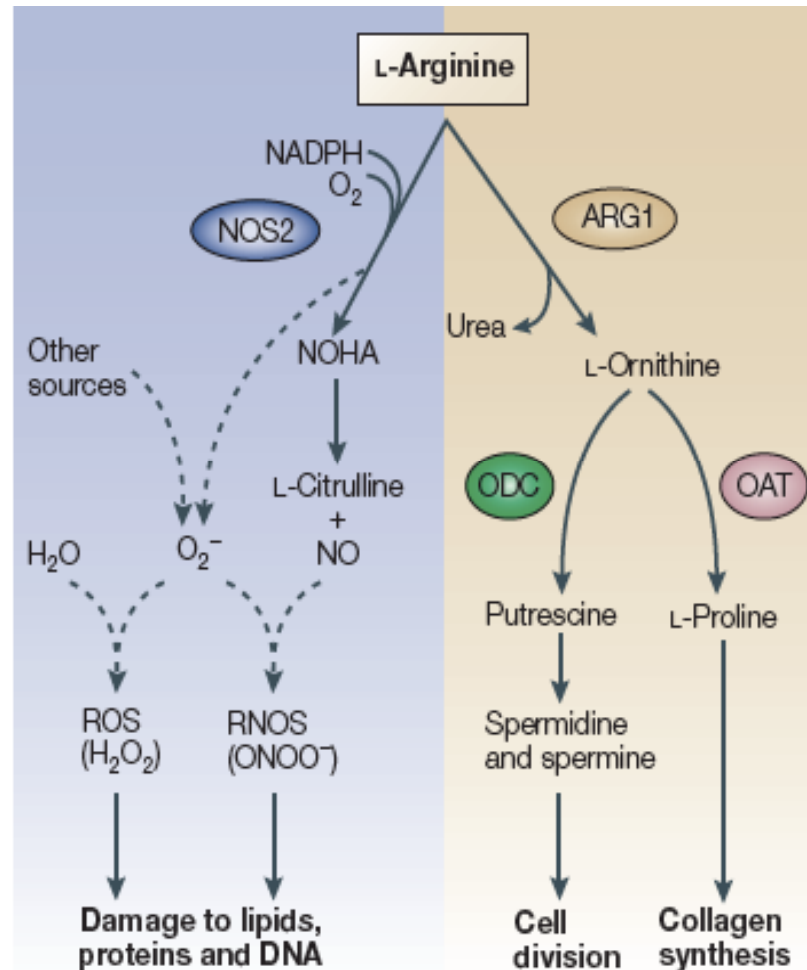


# Arginase inhibitors restore T cell proliferation

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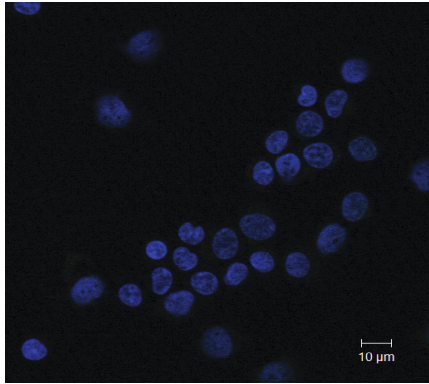
# Arginine metabolism in AML



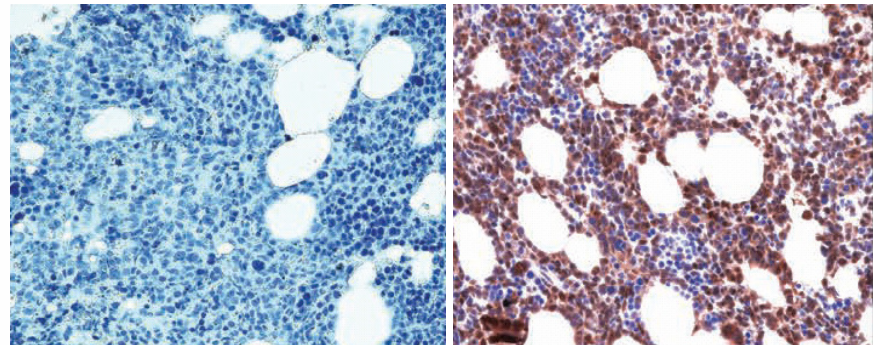
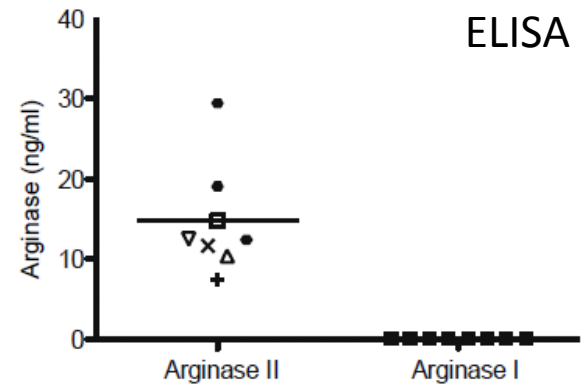
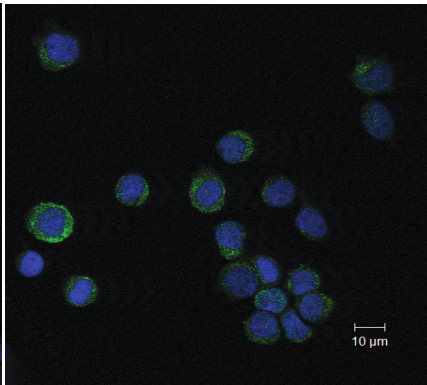
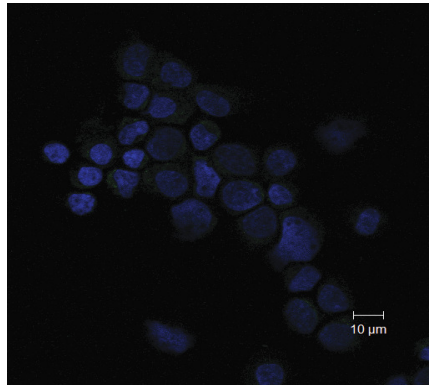
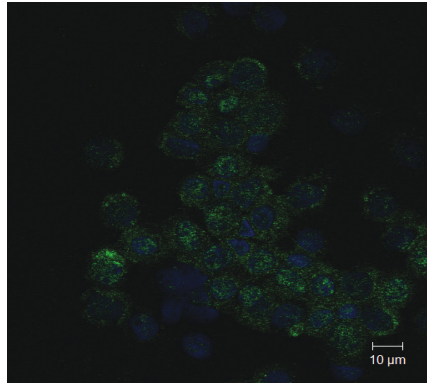
Low/absent expression of ARG1 and iNOS in AML....what's going on?

# Arginase II in AML blasts

Secondary alone

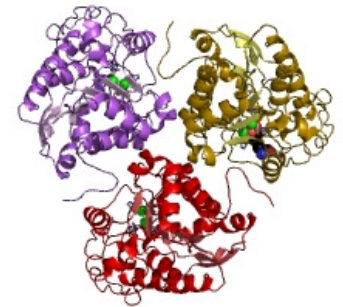


ARGINASE II



# Arginase I vs II

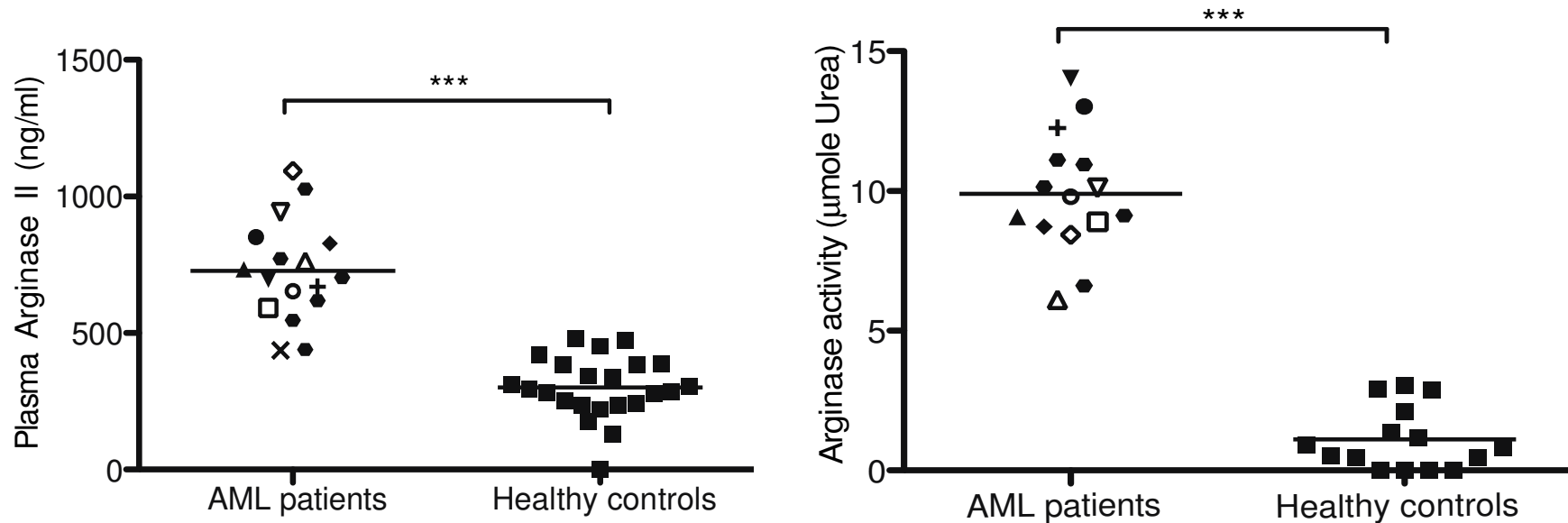
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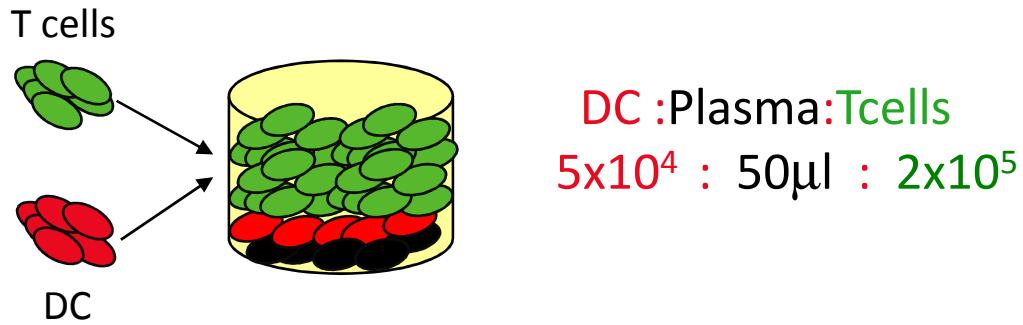
- **ARG I**
  - Chromosome 6
  - Hepatocytes (*Morris et al. 2007*)
  - Cytoplasmic
  - KO in mice is lethal (*Iyer et al. Mol Cell Biol 2002*)
  - Deficiency in humans leads to profound hyperargininemia, neurodisability/toxicity, organ dysfunction (*Schlune et al. Amino Acids 2015*)
- **ARG II**
  - Chromosome 14 (?gene duplication)
  - 60% amino acid sequence homology. 354 aa (*Colleluori et al. 2001*)
  - Tissue localised – renal, neural, endothelial (*Jenkinson et al. 1996*)
  - Mitochondrial (imported via a 22-residue N terminal sequence) (*Morris et al. 1996*)
  - KO in mice is unsymptomatic (mild hyperargininemia) (*Shi et al. 2001*)
  - No human phenotype identified
- Both convert arginine into urea and ornithine
- Not much known about the role of Arginase II in immunity

# AML extends the immunosuppressive microenvironment. ARGII release into the blood

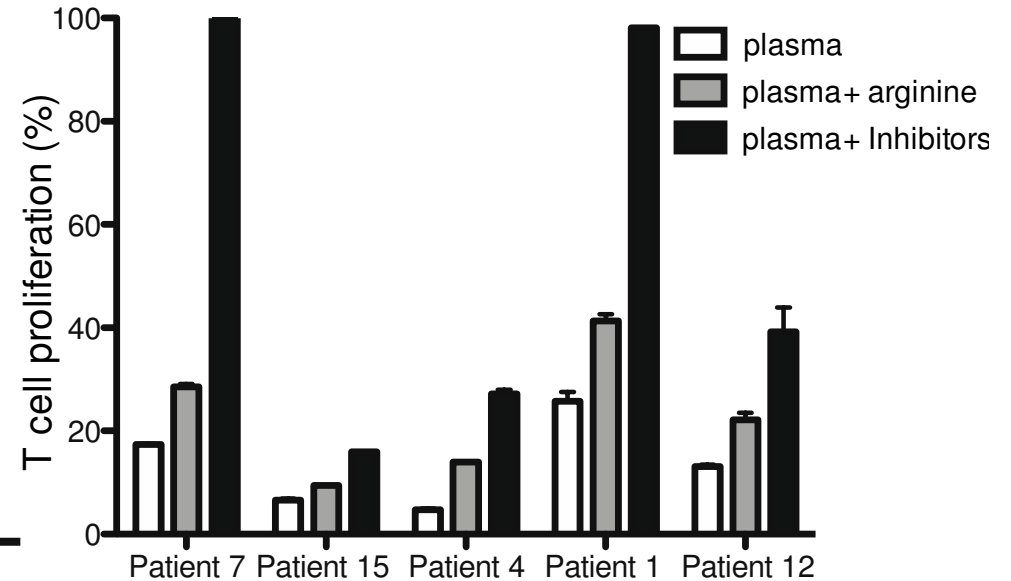
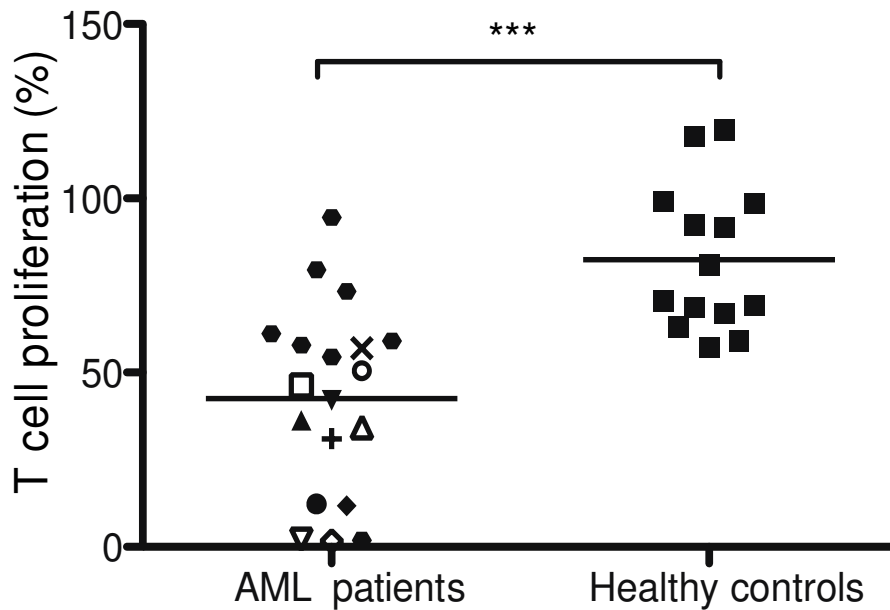
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# T cell inhibition by the blood of AML patients



MLR = Mixed Leukocyte Reaction



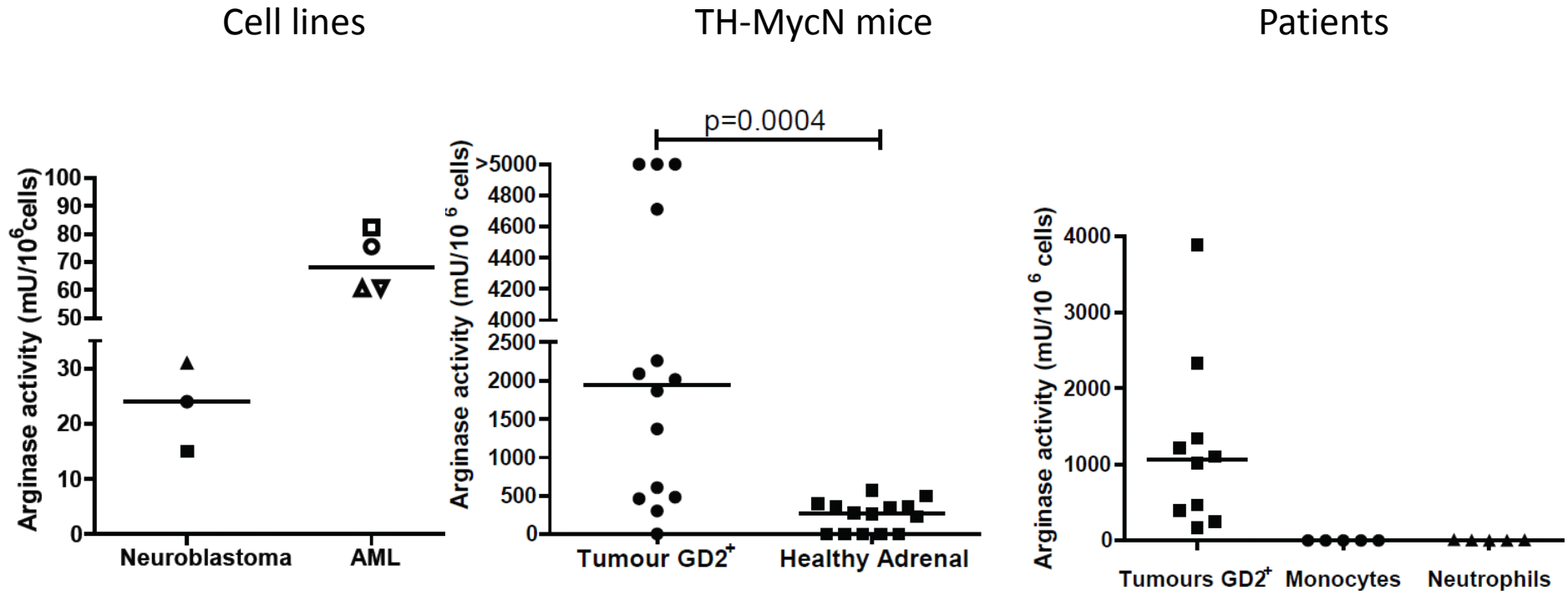
# Summary 1

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- AML is reliant on extracellular arginine and is unable to recycle arginine from precursors
- ARGII expression depletes arginine in the local and systemic microenvironment through expression and release of ARGII
- AML mimics MDSC phenotype

Is this unique to AML?

# Neuroblastoma tumour cells have arginase activity



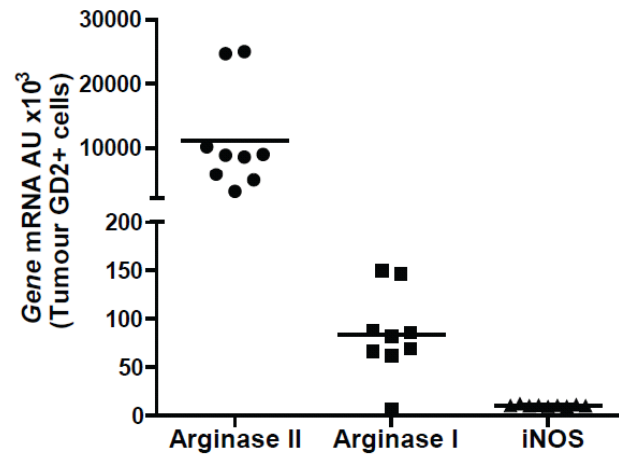
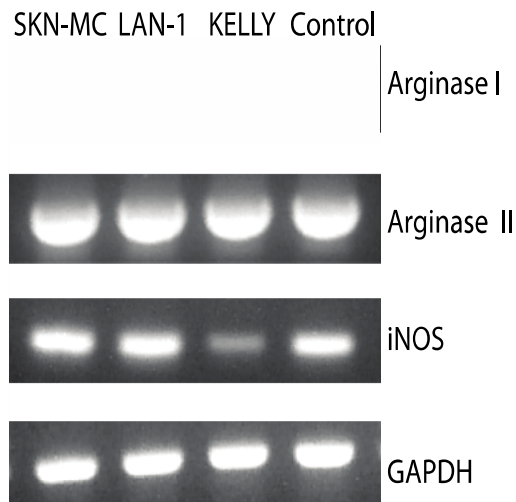


# Neuroblastoma expresses Arginase II

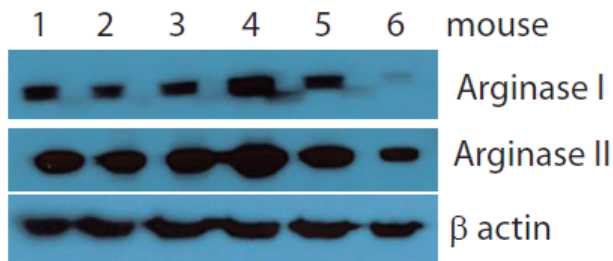
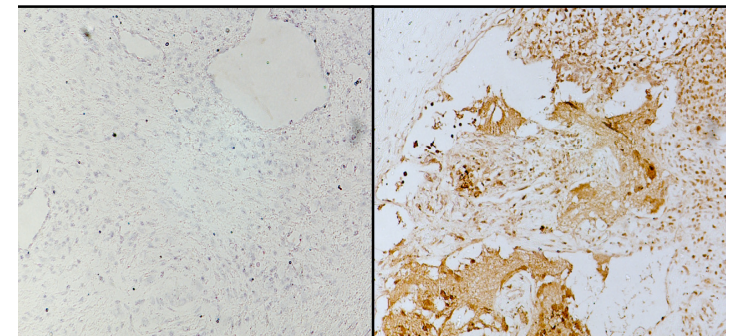
Cell lines

TH-MycN mice

Patients

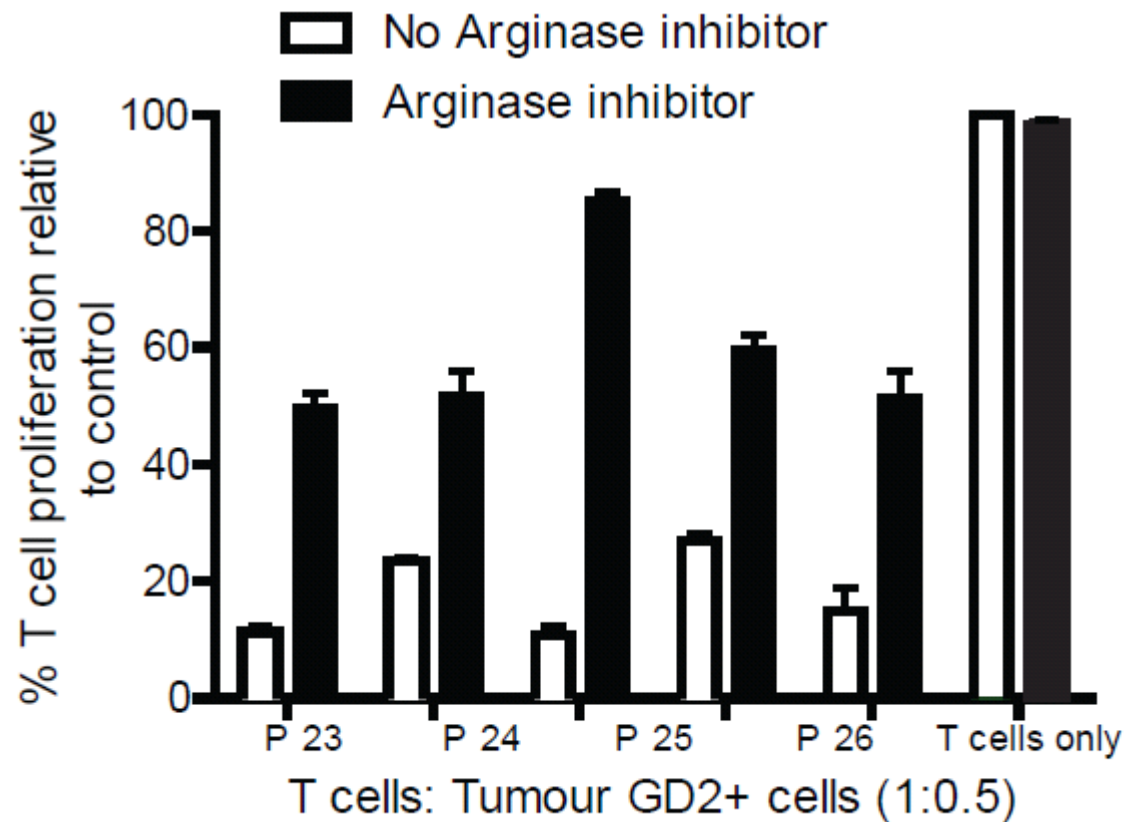


Anti rabbit ab Arginase II

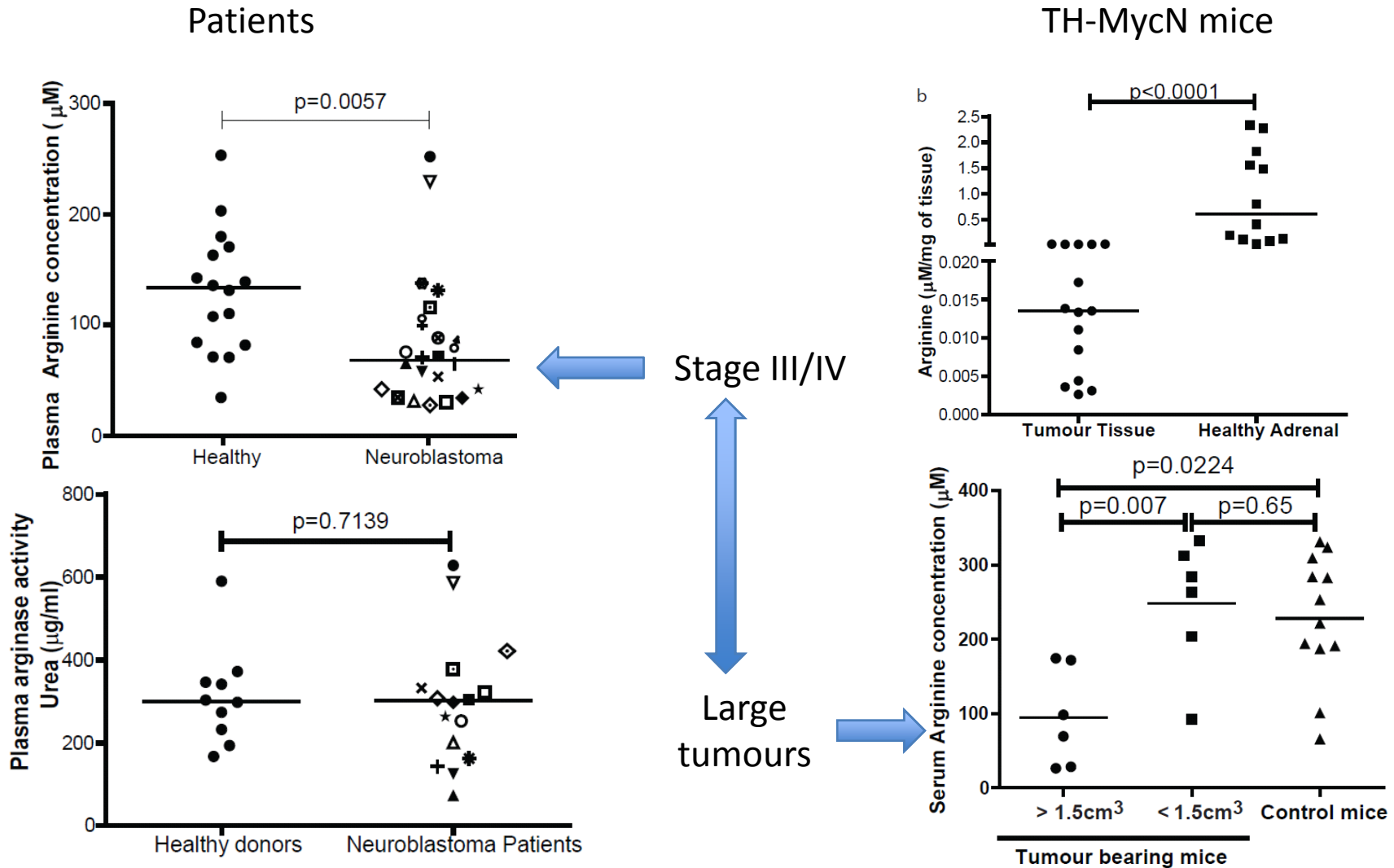


# Resulting T cell suppression

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# Tumour mass lowers plasma arginine

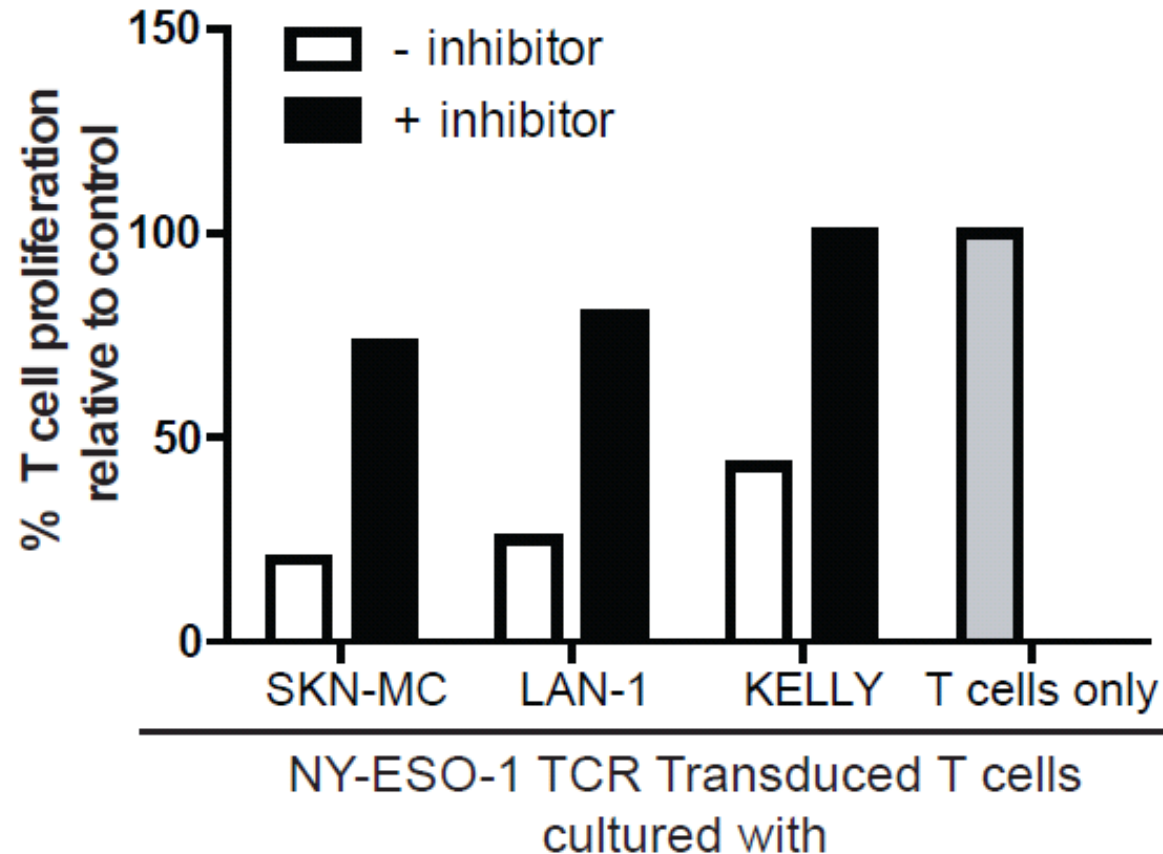


# Impact on immunity

## Antigen specific immunity is impaired

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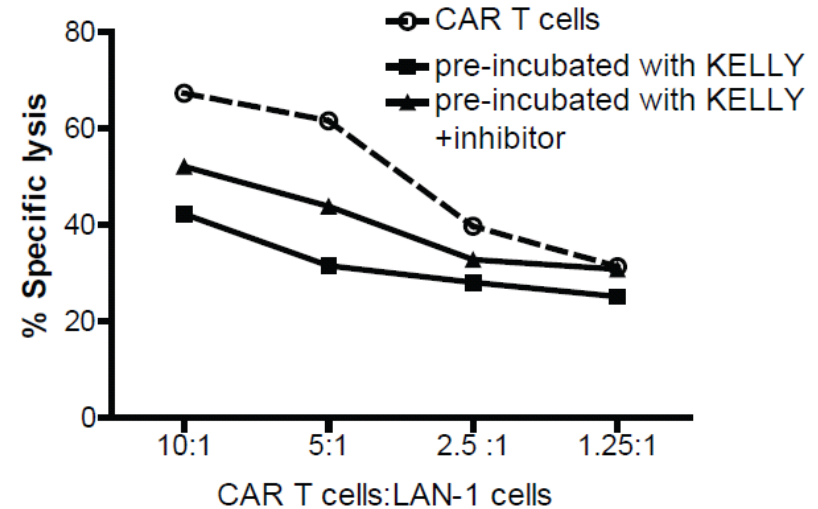
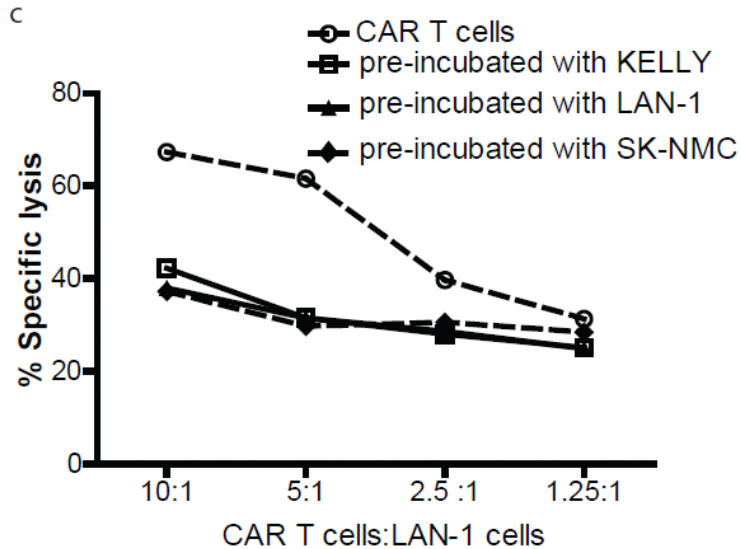
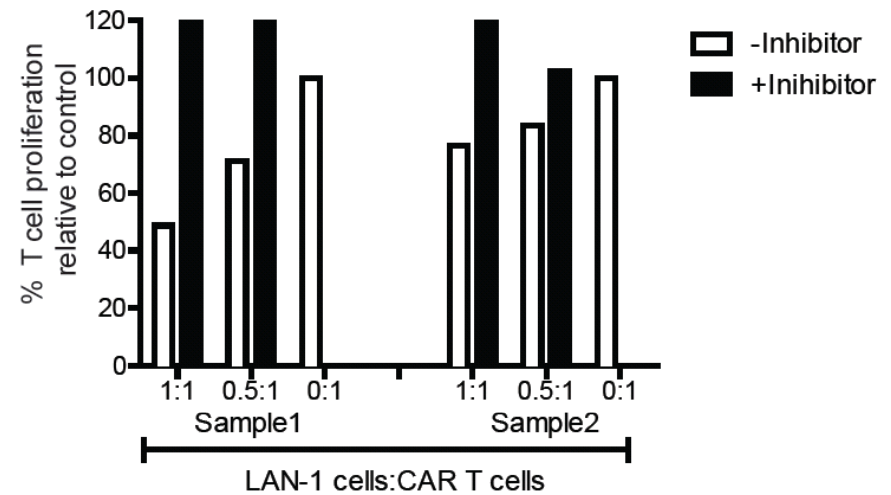
NY-ESO is the most prevalent cancer-testis antigen in NB



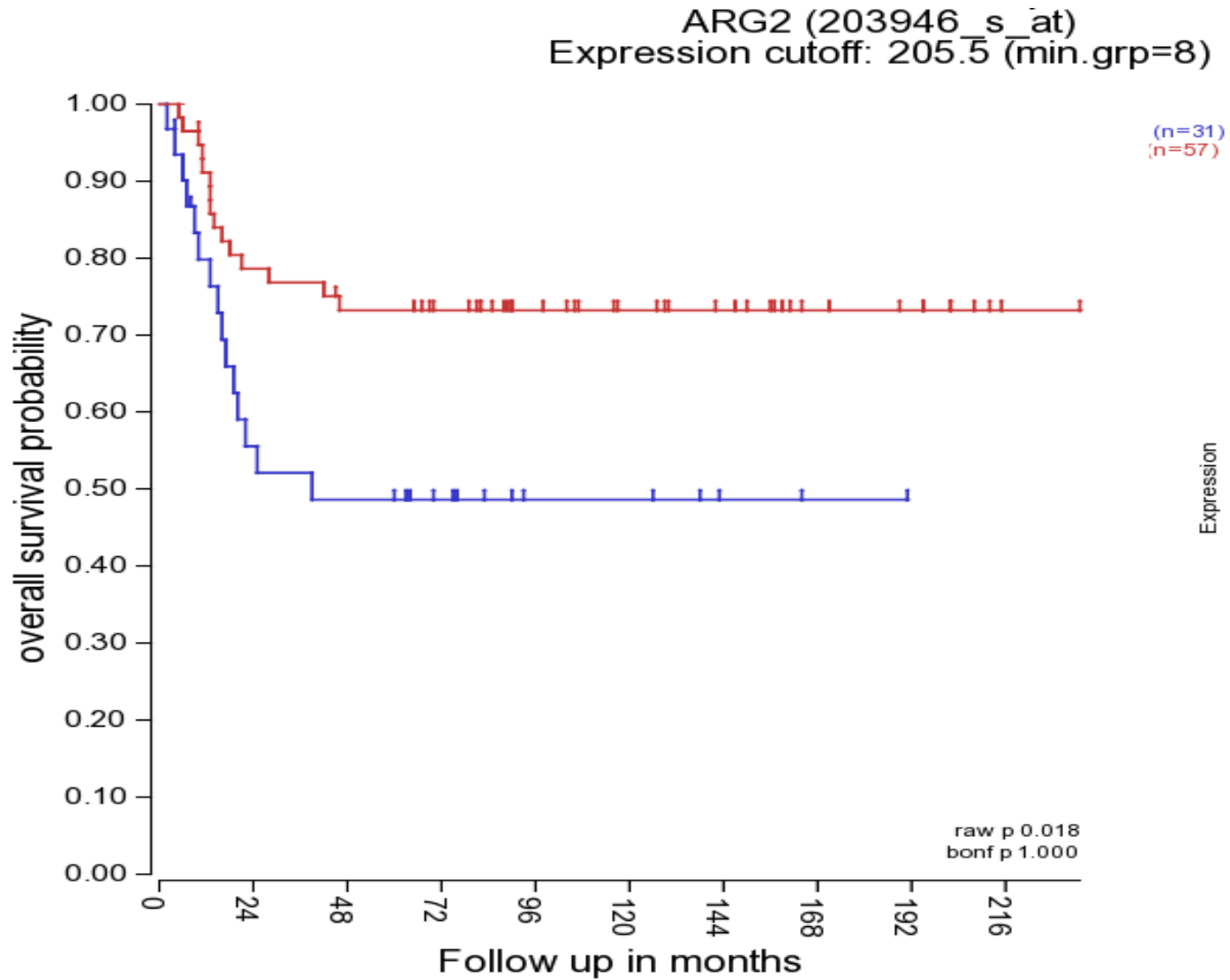
# Impact on immunity

## CAR-T cell function is impaired

Anti-GD2 CAR-T cells - new approach for NB  
 Outcomes limited by failure of sustained CAR-T cell proliferation in patients



# Neuroblastoma ARG2 expression impacts survival



# Summary 2

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- Neuroblastoma expresses ARG2 and depletes local and systemic arginine
  - (Neuroblastoma is auxotrophic for arginine)
- Modulation of
  - Haematopoiesis
  - Myeloid cell immunity
  - T cell immunity
    - Autologous
    - Antigen-specific
    - Engineered CAR T cells
- Associated with a worse prognosis

# Summary 3

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- Tumours import arginine for survival and proliferation
- Lowers local and systemic levels of arginine
- Modulates surrounding myeloid cells to an immunosuppressive phenotype
- Inhibits autologous T cell proliferation
- Suppresses engineered therapeutic T cell approaches



# Thank you

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## University of Birmingham

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## Great Ormond Street

- John Anderson
- Jon Fisher

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- Kate Wheeler
- Jennie Godwin

Staff, patients and parents

