Investigational Medicinal Products (IMP)

Nucleic Acids, Genes, Cells and Peptides for Immune Gene Therapy of leukaemia, solid tumours and in regenerative medicine

Dr Siamak Salehi MSc, MD, PhD, PG Dip

Senior Lecturer in Transplantation

Liver Studies
Division of Hepatology and Transplantation
King's College Hospital NHS Trust
King's Health Partners
London, UK

Professor Farzin Farzaneh D.Phil., FRCPath., FRSB

Chair of Molecular Medicine, King's College London

Division of Haematological Medicine
Honorary Consultant in Specialist Medicine
King's College Hospital NHS Trust
QP – Guy's and St Thomas' Hospital NHS Trust
Great Ormond Street Hospital NHS Trust

Licenced GMP facilities for the production of Cell & Gene based Investigational Medicinal Products (IMP) since 2001

- **Cancer Vaccination – gene modified cells**
- **CAR T Cell therapies – autologous**
- **CAR T cell therapies – allogeneic**

- Lysosomal storage disorders
- Metabolic disorders
- Neuromuscular disorders
- Transplant associated virus reactivations

We have manufactured the largest number of viral vectors for clinical trials in Europe

ADCC :
Antibody Dependent Cellular Cytotoxicity

New GMP Facility, enabling parallel production of 2 different vectors, to go into production in Q4 2019.

- Potentially doubling the manufacturing capacity

Small-scale manufacturing facility for rapid transition of large numbers of gene therapy products to the clinic

……plasmids to clinical viral vectors <6 months!

- **Peptide library (hTERT) therapeutic vaccine against solid tumours**
- **BiTE antibody manufacture for Phase-I/II trial in 2020**
- **Small scale AAV in 2020**
- **DNA vaccines Brain Tumours: in clinic**

- Gene Editing: Crisper
- BMSC for MS
- Hepatocyte /Islet Transplant

CRF

NIHR/Wellcome King's Clinical Research Facility (CRF)

CTF Clinical Trials Facility

- 11 x Infusion/phlebotomy chairs
- 8 x Ward Beds (including 1 x isolated side room)
- 2 x Renal Dialysis facilities
- 4 x Clinical examination rooms
- 1 x Quiet lounge
- Fully fitted nutritional kitchen
- Sample processing room with centrifuge, fridge and -20^{0}C and -80^{0}C freezer
- Networked to KCL, KCH and SLaM Trusts
- Patient recreation/waiting area and courtyard
- Two car parking spaces (needs assessed)

- Administrative centre

EMF Experimental Medicine Facility

Ground Floor

- MRI: 3T functional MRI (fMRI) scanner
- 2x HDU beds
- 4x ward beds (including 2 x isolated side rooms)
- Secure Cryostore
- IT data room

1st Floor

- Main reception
- 4 x Interview rooms with video recording and observation facility
- 2 x Clinical Consultation rooms
- 6 x Infusion/phlebotomy chairs
- 2 x EEG recording rooms
- Virtual Reality suite
- Physiotherapy suite
- Sample processing + storage area
- Patient recreation area

CTU Cell Therapy Unit

GMP facility with 3 process suites:

- Grade B suite with closed processing in 1
- Grade A isolator: Hepatocytes & Islets cell isolation Grade D suite with closed processing in 3 Grade A isolators: Cell Therapy (BMT & MSC)
- Grade D suite with closed processing in 2 Grade A isolators: Cell & Gene Therapy

Additional controlled rate freezer room for cell freezing,
cold pack, prep and waste treatment

Common area & sample handling (outside CTU): QC lab, changing rooms, meeting room, write up & staff refreshment and GMP storage facility

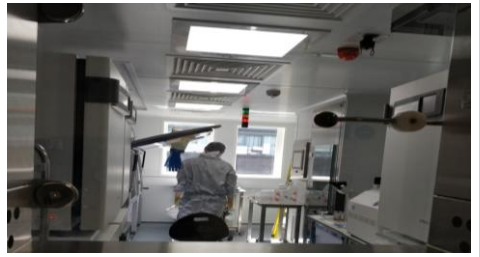
King's Advanced Medicine Programme
Investigational Medicinal Products (IMP)

Cell Therapy Unit (CTU)

GMP facility with three process suites contains:

* Grade B suite with closed processing in 1 Grade A isolator: Hepatocytes and Islets cell isolation
* Grade D suite with closed processing in 3 Grade A isolators: Cell Therapy (BMT & MSC)
* Grade D suite with closed processing in 2 Grade A isolators: Cell & Gene Therapy

Cell Therapy Suite (CTS)
(GMP Production Facility)



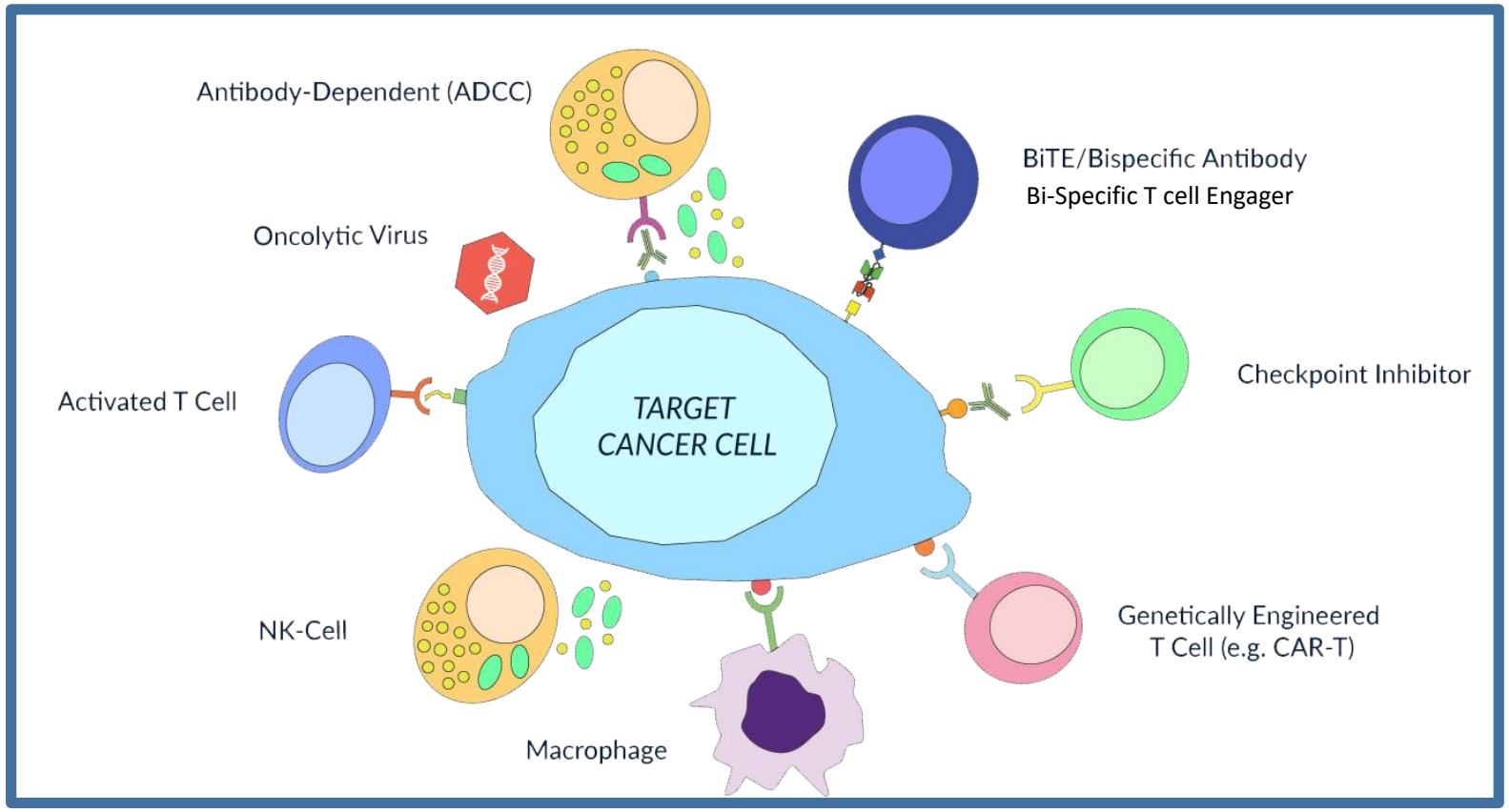
* GMP production facility since 2001
* Licensed by the Human Tissue Authority (HTA) for the procurement, storage, human use, import and export of cell therapy based products.
* licensed by the Medicines and Healthcare products Regulatory Agency (MHRA)
* "Specials License" (MS) for the production of cell and gene therapy products for their off-trial clinical use. Both the MS and MA IMP licenses have import and export permits.

5 pillars of cancer treatment

1- Surgery
2- Radiotherapy
3- Chemotherapy
4-Targeted therapy

5- Immuno-oncology (IO) looks set to become the fifth pillar of cancer treatment

Immune System and targeting cancer



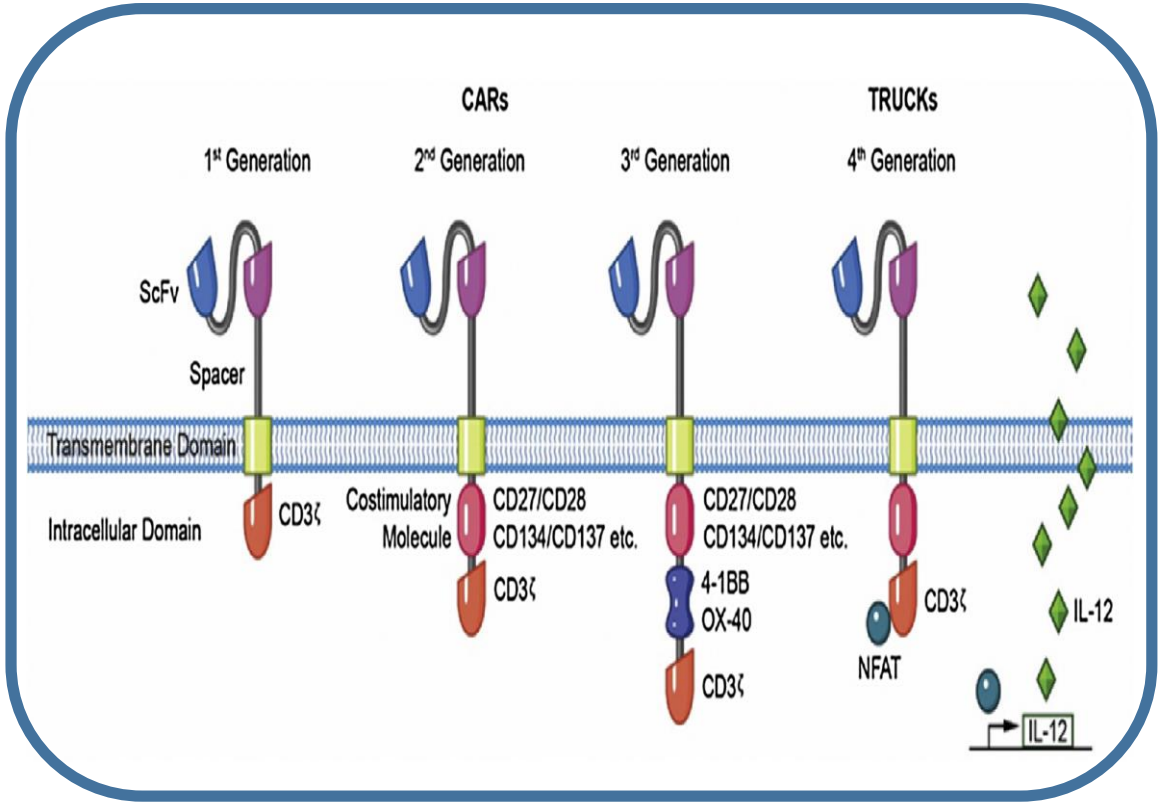
Chimeric Antigen Receptors (CARs) for Immune Therapy of Cancer

T cell activation requires the stimulation of multiple receptors – *i.e.* multiple keys need to be turned on simultaneously to enable antigen specific toxicity

CARs utilise synthetic receptors that provide several key signalling molecules as a result of encounter with cancer associated antigens

Better CAR T cells against solid tumours need:

- **Enhanced trafficking and tumour infiltration**
- **Enhanced functional efficacy**
- **Increased resistance to tumour mediated immune suppression**

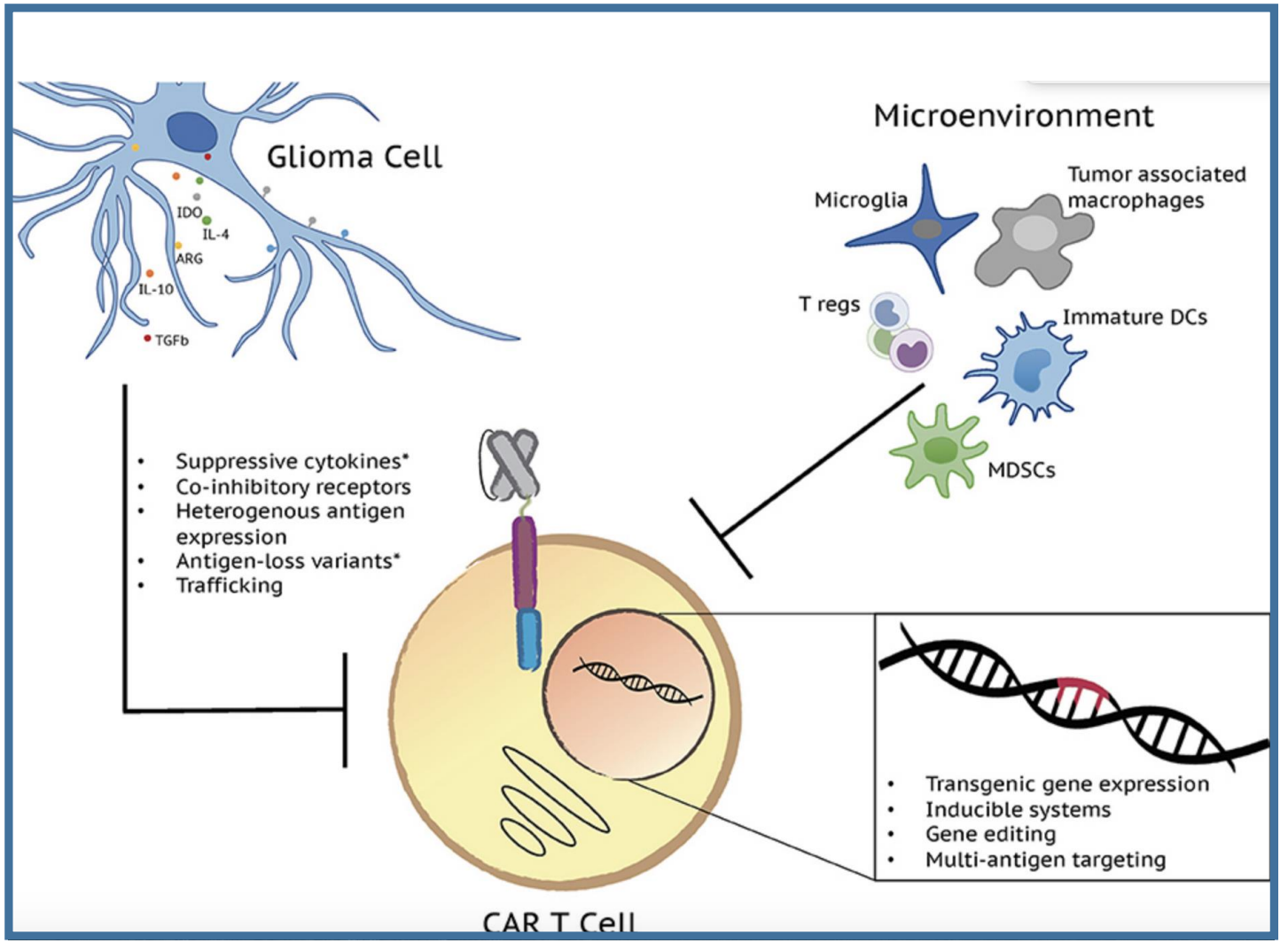


Aaron J. et al. Chimeric antigen receptor (CAR) T cell therapy for malignant cancers :Summary and perspective. J. of Cellular immunotherapy, Volume 2, Issue 2, November 2016, Pages 59-68

The **fourth generation** of **CAR-T cells** is known as **T-cells** redirected for universal cytokine-mediated killing (TRUCKs)

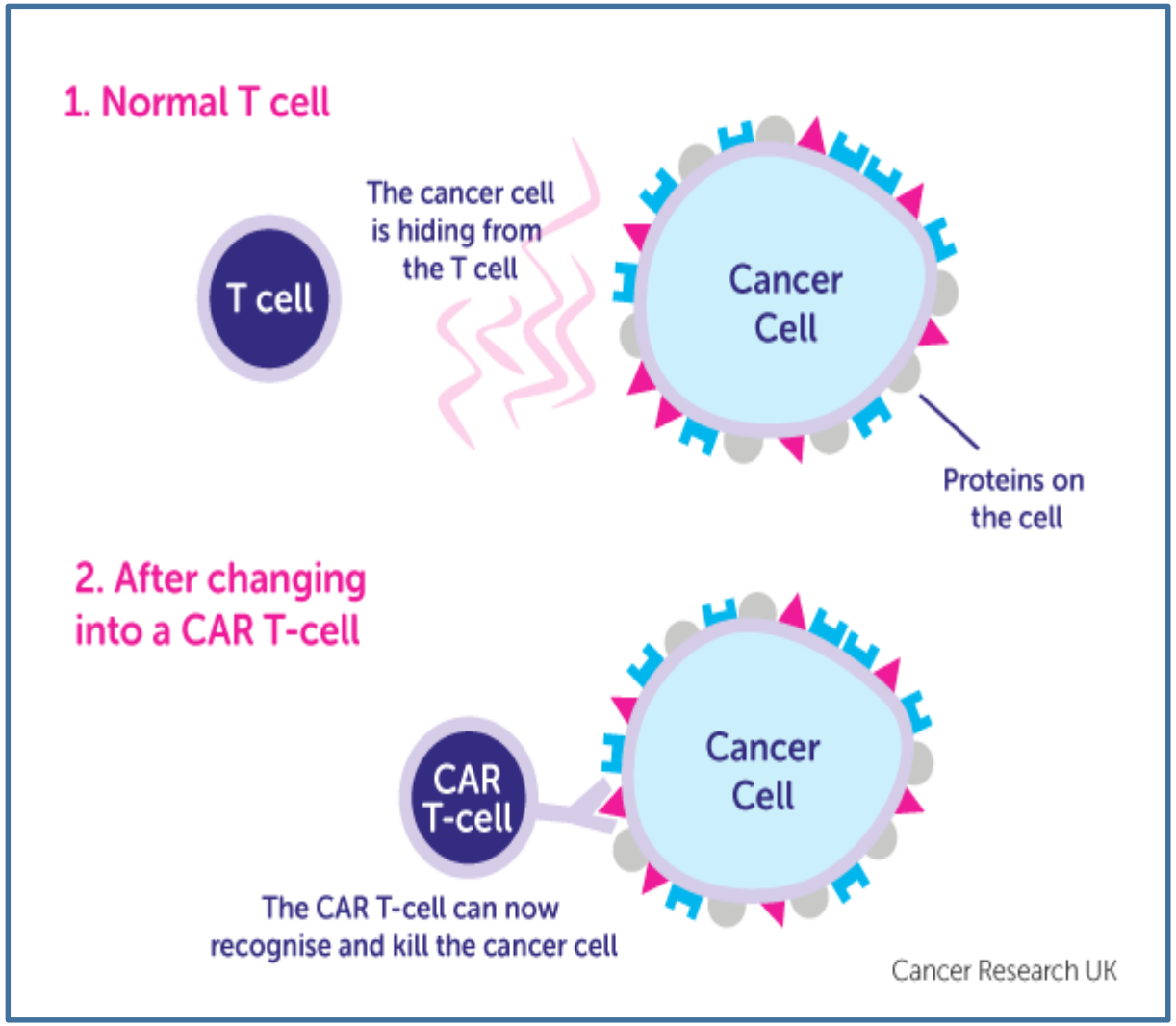
Designer T cell lines for optimal safety, efficacy and standardisation of CAR T cell therapies

Next Gen



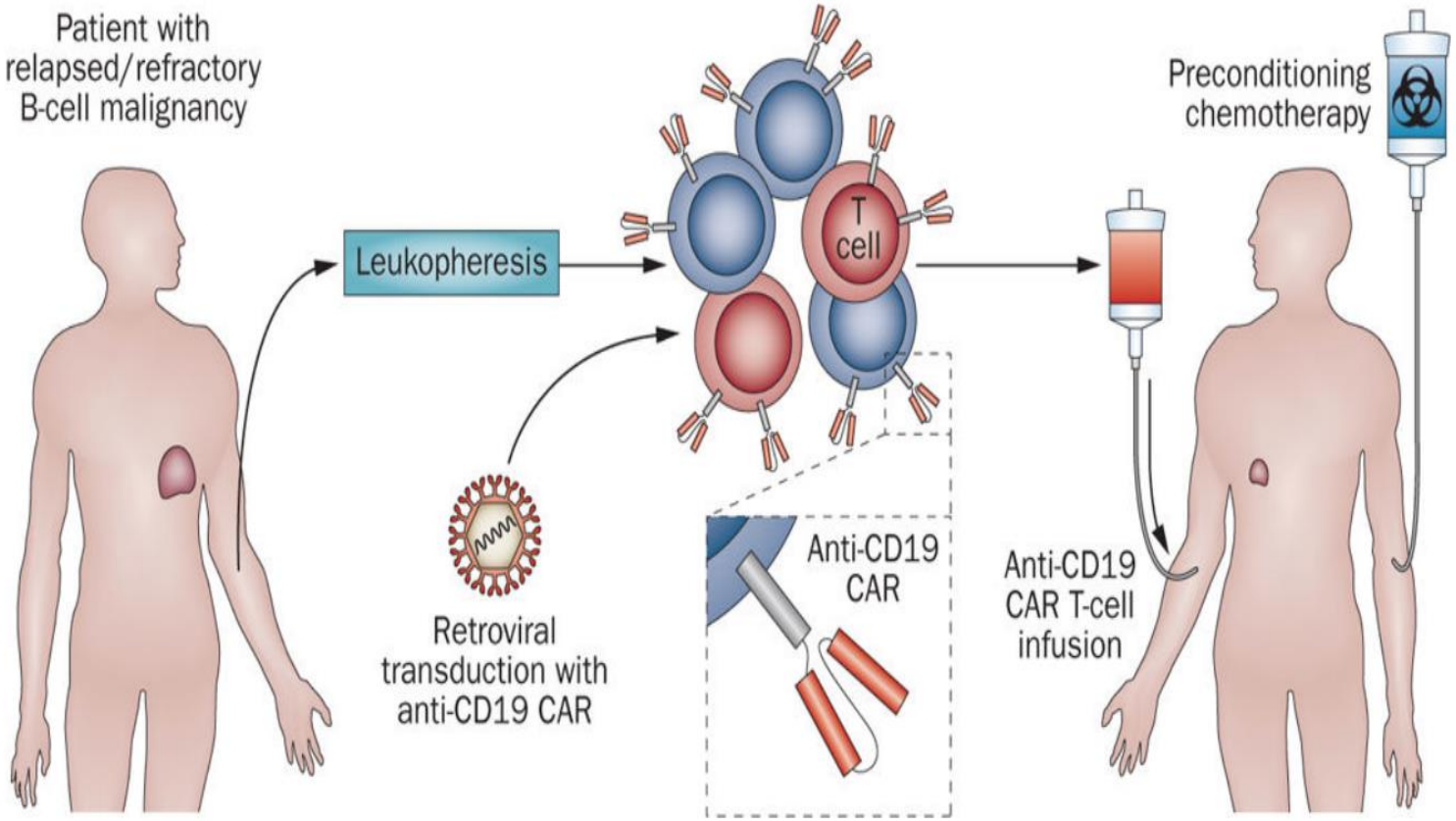
Christopher T. Petersen and Giedre Krenciute*

Next Generation CAR T Cells for the Immunotherapy of High-Grade Glioma. Front. Oncol., 26 February 2019


1. Normal T cell
T cell
The cancer cell is hiding from the T cell
Cancer Cell
Proteins on the cell
2. After changing into a CAR T-cell
CAR T-cell
Cancer Cell
The CAR T-cell can now recognise and kill the cancer cell
Cancer Research UK

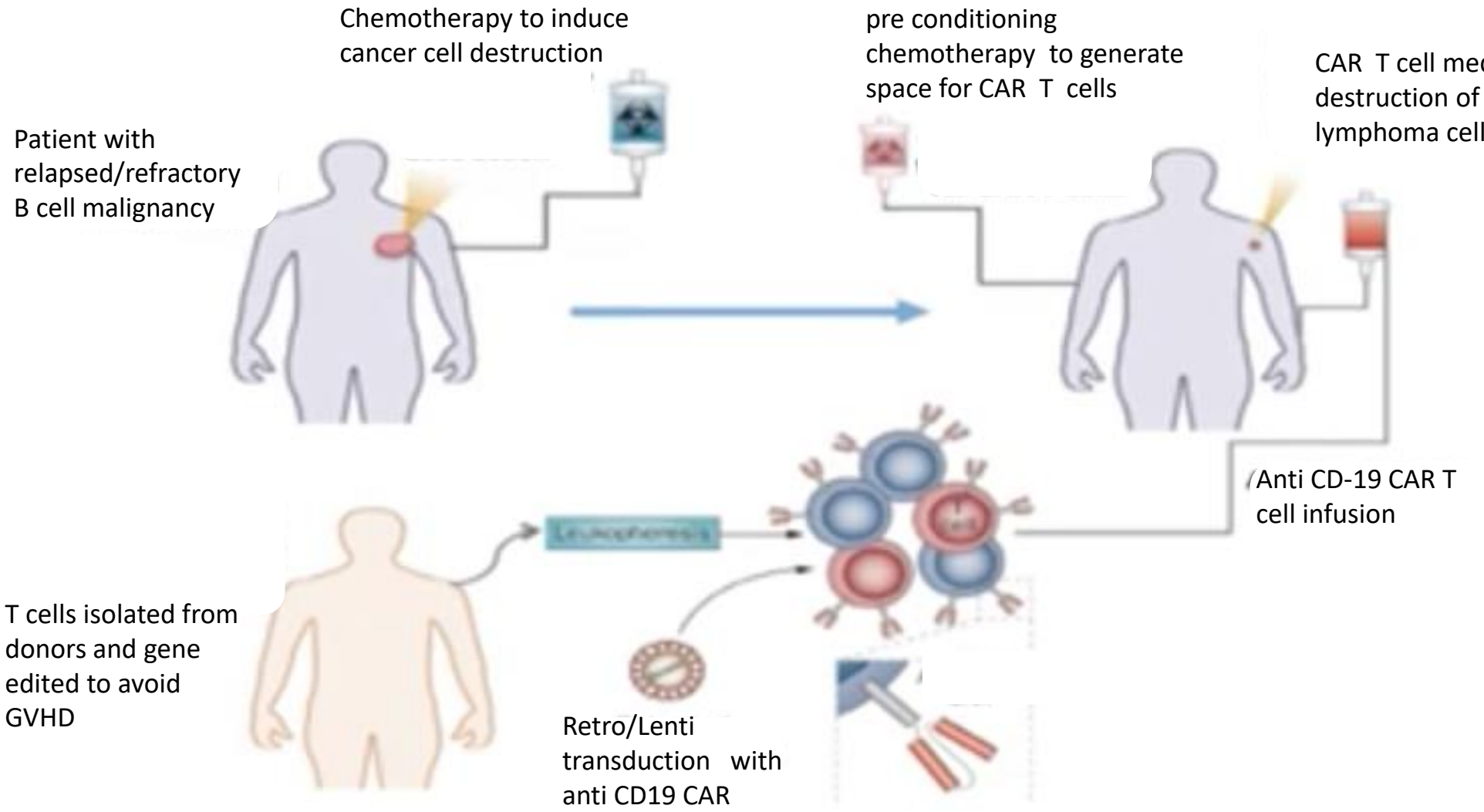
Treatment of patients with B-cell malignancies using autologus anti-CD19 CAR T cells

- **Cure Rate: >80%**
- **Emergence of escape clones**
- **Serious CRS**



Nature Rev Clin Oncol. 2014 December ; 11(12): 685–686.
doi:10.1038/nrclinonc.2014.190.

Treatment of patients with B-cell malignancies using allogenic anti-CD19 CAR T cells



Targets of CAR-T Cell Therapy

KING'S College LONDON

CAR-T-Cell targets for the treatment of heamatological cancers

Target	CAR structure	Malignancy
BCMA	CD3ζ and 41BB	MM
CD19	CD3ζ and CD28; CD3ζ and 41BB KIR2DS2 and DAP12-	Lymphoma; Leukemia
CD22	CD3ζ and CD28	FL; NHL; DLBCL; ALL
CD20	CD3ζ; CD3ζ and 41BB-	CD20positive malignancies
CD138	CD3ζ and 41BB	MM
CD33	CD3ζ and 41BB	AML
CD123	CD3ζ and CD28	AML
CD19 CD20	CD3ζ and 41BB	Leukemia; Lymphoma
CD19 PSMA	CD3ζ and CD28 PD-1 or CTLA4	Leukemias
FITC-CD19 Ab	CD3ζ and CD28	CD19 positive cancers
Igκ	CD3ζ and CD28	CLL
LeY	CD3ζ and CD28	AML
ROR1	CD3ζ and 41BB	CLL; SLL

CAR-T-Cell targets for the treatment of solid tumours

Target	CAR structure	Malignancy
Biotin	CD3ζ, CD28 and 41BB	EGFRvIII positive cancer
CD171	CD3ζ and 4-1BB; CD3ζ, CD28 and 4-1BB	Neuroblastoma
EGFRvIII	CD3ζ and 41BB CD3ζ and ICOS-	Glioma
FAP	CD3ζ and CD28 KIR2DS2 and DAP12-	Mesothelioma; Lung cancer
FR	CD3ζ and CD27	Ovarian cancer; Breast cancer
Glypican-3	CD3ζ, CD28 and 41BB	Hepatocellular carcinoma
HER2	CD3ζ and CD28	HER2 positive cancer; Sarcoma
HER2 MUC1	CD3ζ and CD28	Breast cancer
HER2 IL13Rα2	CD3ζ and CD28	Glioblastoma
IL13Rα2	CD3ζ; CD3ζ and 41BB CD3ζ and CD28 CD3ζ, CD28 and 41BB CD3ζ, CD28 and OX40-	Glioma
Mesothelin	CD3ζ; CD3ζ and CD28 CD3ζand 41BB CD3ζ and ICOS KIR2DS2 and DAP12-	Mesothelioma; Pancreatic cancer; Non-small cell lung cancer
Mesothelin CD19	CD3ζand 41BB	Pancreatic cancer
MUC1	CD3ζ and 41BB	MUC1 positive solid tumor

https://www.creativebiomart.net

Current UK CAR T-Cell programme (Treatment not trial)

Children and young people : B cell ALL

- Newly diagnosed children or young people up to age of 25 whose leukaemia hasn't gone away with 2 cycles of treatment
- Relapsed following a stem cell or bone marrow transplant
- Their disease has relapsed twice or more
- Remission after first cycle of treatment but relapse after a period of time and resistant to second cycle of chemotherapy
- Relapse once but they can't have a stem cell transplant because either they aren't well enough, or they don't have a donor

- Tisagenlecleucel (Kymriah)
- £282,000 per patient
- 15-30 patients a year.

Adults : For 2 types

* Diffuse large B cell lymphoma (DLBCL)
* Primary mediastinal B cell lymphoma

* It is for those adults whose lymphoma has continued to grow or relapsed following at least 2 treatments.

So this treatment is only suitable for a small number of children and young people, and around 200 adults each year. It is not used as a treatment outside of clinical trials for other types of cancer in children or adults.

- Axicabtagene-ciloleucel (Yescarta)
- £300,000 per patient
- 200 patients a year

centres providing CAR-T for ALL (children and young people up to the age of 25)

1. Great Ormond Street Hospital
2. University College London Hospital
3. **King's College Hospital**
4. University Hospitals Bristol NHS Trust
5. The Christie NHS Foundation Trust
6. Manchester Royal Infirmary
7. Royal Manchester Children's Hospital
8. Queen Elizabeth Hospital (Birmingham)
9. Great Northern Children's Hospital (Newcastle)

centres providing CAR-T for adults with large B-cell lymphoma:

1. University College London Hospital
2. **King's College Hospital**
3. University Hospitals Bristol NHS Trust
4. The Christie NHS Foundation Trust
5. Manchester Royal Infirmary
6. Queen Elizabeth Hospital Birmingham
7. Newcastle Hospitals NHS Foundation Trust

Non-Hodgkin Lymphoma >85%

Overall response rate in relapse and refractory NHL

Acute Lymphoblastic Leaukemia >90%

Overall response rate in relapse and refractory ALL

Multiple Myeloma >80%

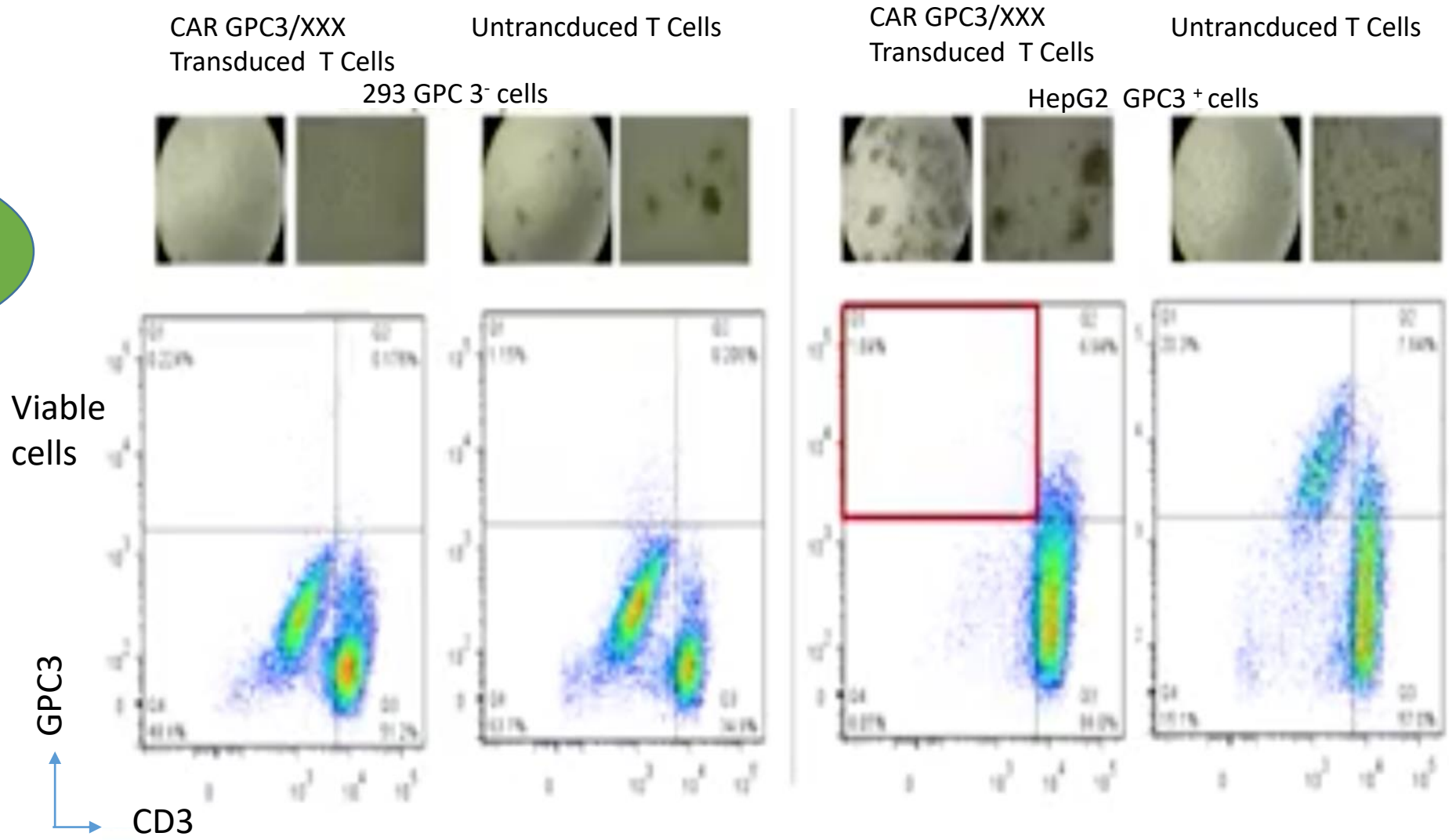
Overall response rate in relapse and refractory MM

CAR T –Cell for Hepatocellular Carcinoma (HCC)

CAR GPC3 / XXX

Lysis of GPC3 + cells by CAR GPC3/XXX

New generation of Bi-Specific CAR for HCC



CAR-T-Cell Therapy Side Effects

- **Cytokine-Release Syndrome (CRS)**
- **Neurologic Toxicities**
- **B-Cell Aplasia (On Target-Off Tumour)**
- **Tumour Lysis Syndrome (TLS)**
- **Anaphylaxis (Life-threatening Allergic Reaction)**
- **Relapse (poor CAR T cell persistence or emergence of $CD19^-$ clones)**

Current work

- **Targets**
- **Cell Type/Resistance**
- **Safety Modifications**

Targets
- Present on Tumour cells not normal cells
- Persistent
- Desired affinity

Resistance
- Ag Escape

Additional targets(CD19,20,22)

Bi-Specific CAR

Cell Type
- BM stem cell transduction
- Modification of selected subset of T Cells

The CARPALL trial (Next Generation CAR T Cells)

- novel CD19CAR with a new scFv (CAT) lower affinity than FMC63
- Increased proliferation and cytotoxicity in vitro /enhanced proliferative and in vivo antitumor activity
- 12/14 patients with relapsed/refractory paediatric B cell achieved molecular remission.
- Persistence in 11 of 14 patients.
- Low toxicity with no severe CRS.

Nature Medicine 02 September 2019 (Sara Ghorashian)

First
CAR-T-Cell Therapy patient in the World

Layla Richards
GOCH London

First NHS Patient
CAR-T-Cell Therapy

Yuvan Thakkar,
GOSH London

- **First person in the world to receive CAR-T-Cell Therapy**
  Received UCART19, a universal, donor-derived CAR T-cell.
- **Achieved remission after UCART19 and proceeded to allogeneic hematopoietic stem cell transplant**

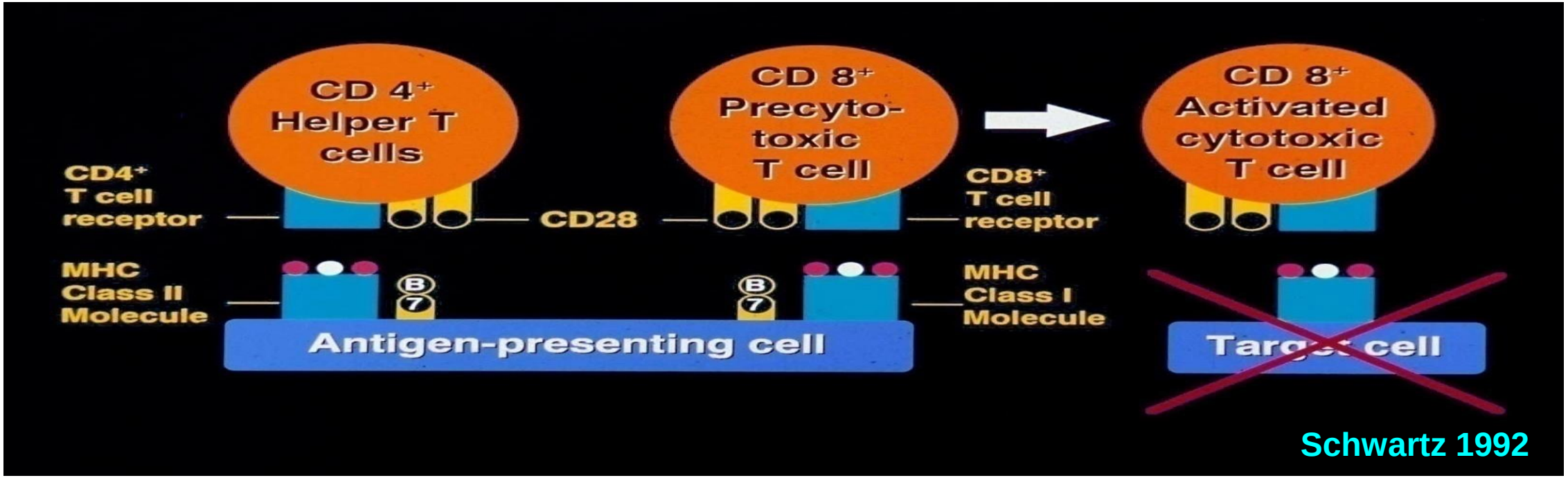
* Kymriah is used

- **Use of site directed endonucleases (TALEN) to achieve:**
  CD52 deletion hence resistance to Campath-an anti CD 52 MoAb
- **Presence of CD20 target epitope for Rituximab mediated in-vivo depletion of CAR T Cells in case of adverse effects**

CD80 & cytokine mediated immune gene therapy of AML

- Autologous leukaemia cell vaccines

Professional antigen presenting cells

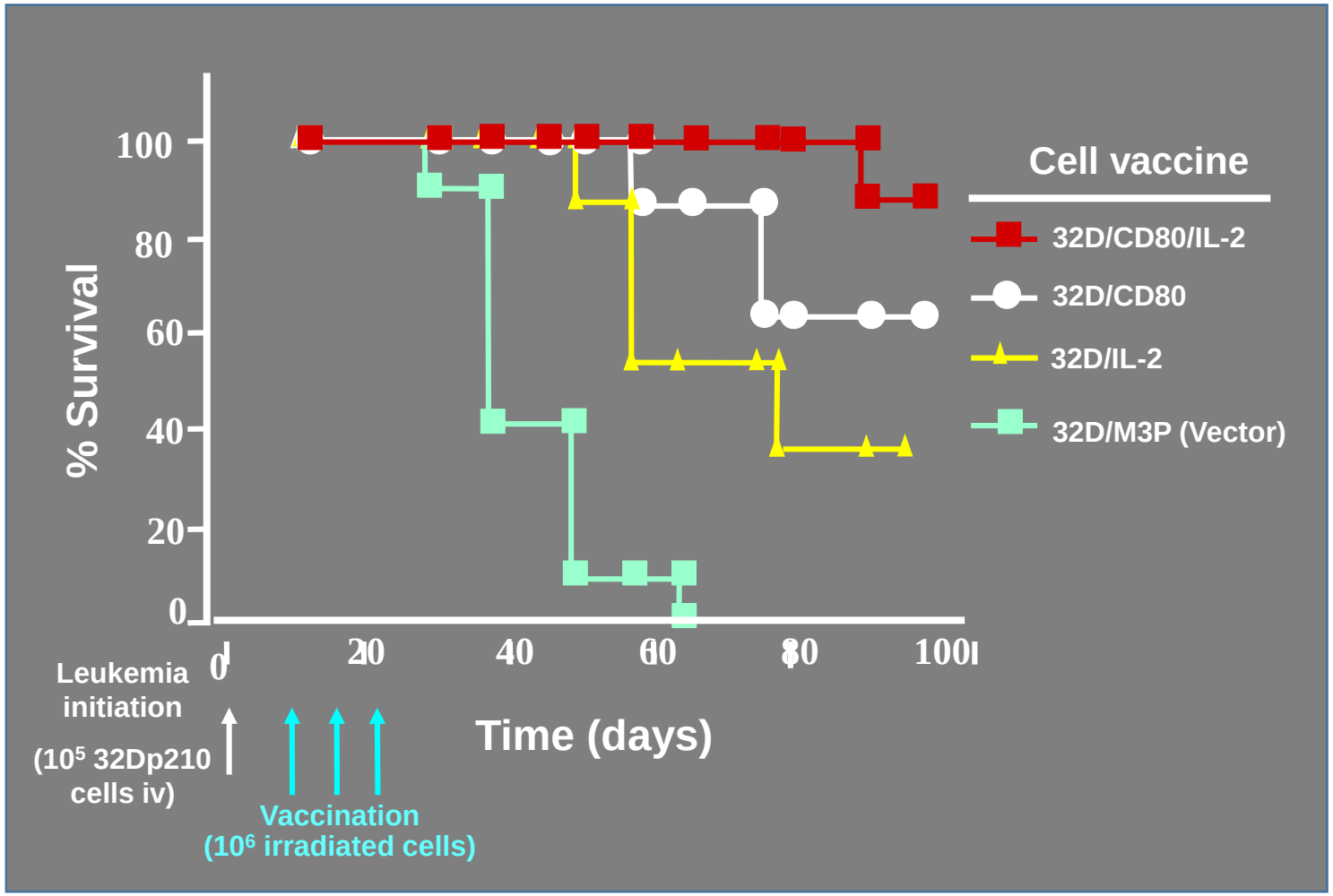


Acute Myeloid Leukaemia (AML)

- AML blasts express both HLA class-I, and class-II
- Express AML associated antigens (WT1, PRAME, GP250, *etc*)
- Share common lineage with APCs – efficient antigen presentation
- Express many surface markers present on DC, including CD86 (B7.2) – but not CD80 (B7.1) !

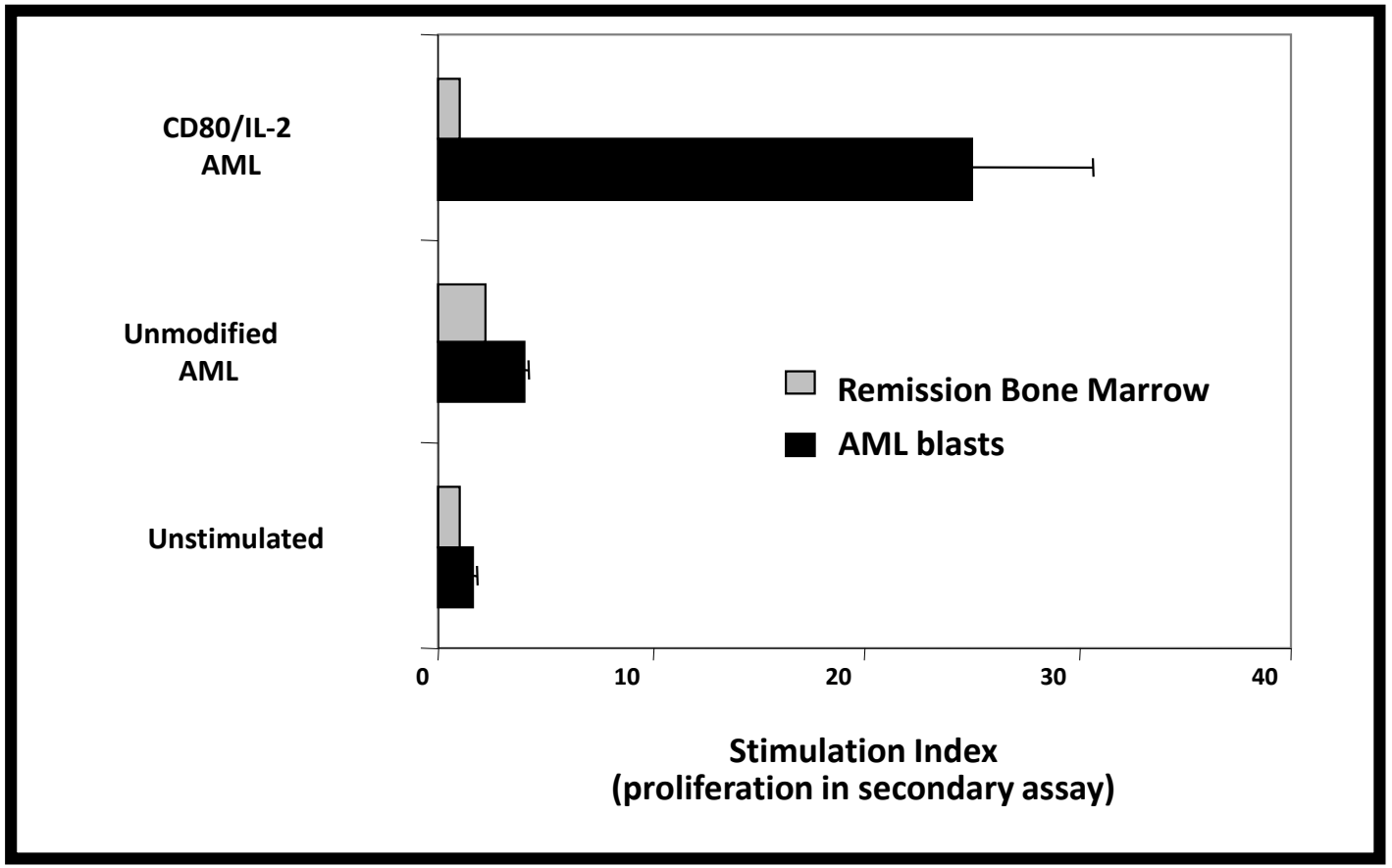
Rejection of established mouse leukaemia, by vaccination with leukemia cells expressing CD80 (B7.1) and IL-2

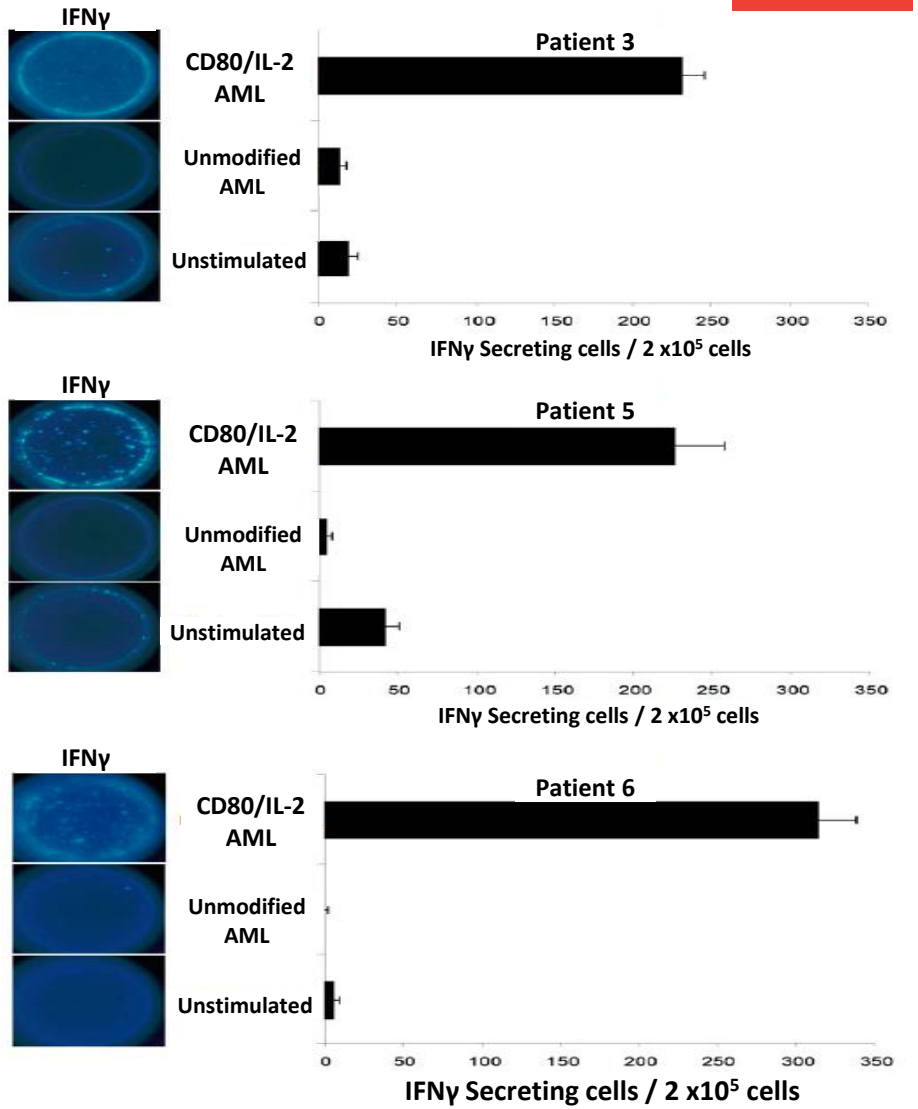
In mouse leukaemia and solid tumour models, vaccination with tumour cells that are modified to express CD80 (B7.1) and IL-2, induces immune mediated tumour rejection.



Specificity of the *in vitro* stimulated T cells

- Unmodified secondary targets

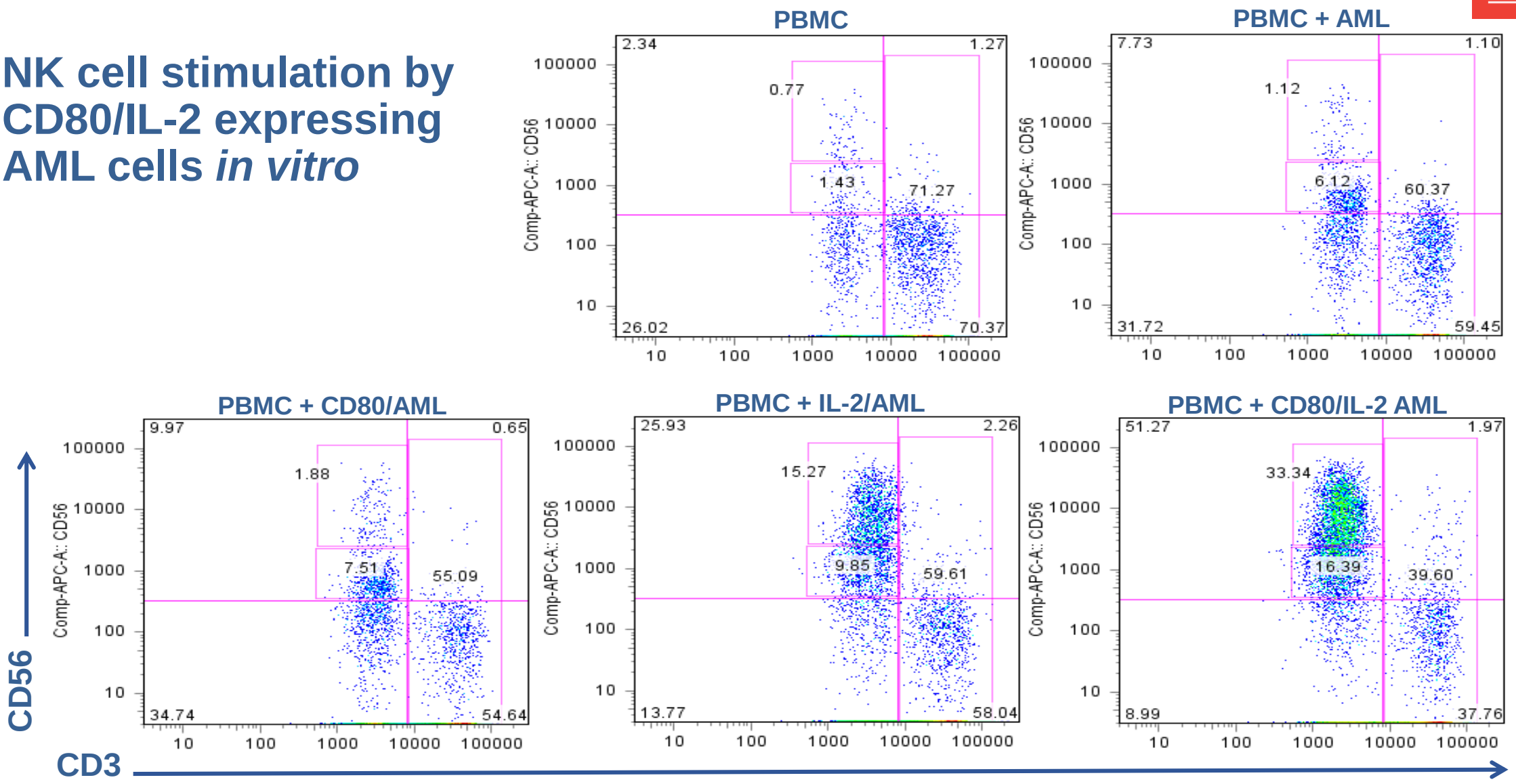




Greater specificity of the CD80/IL-2 stimulated T cells against AML blasts, than the remission CD14⁺ normal bone marrow cells

Hardwick N *et al* (2010). *Cancer Immunol. Immunther.* 59(3): 379-88.

NK cell stimulation by CD80/IL-2 expressing AML cells *in vitro*



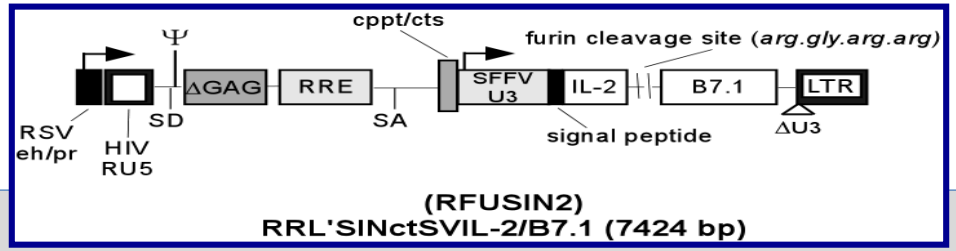
Ingram W *et al* (2009). *Br J Haematol.* 145: 749-60.

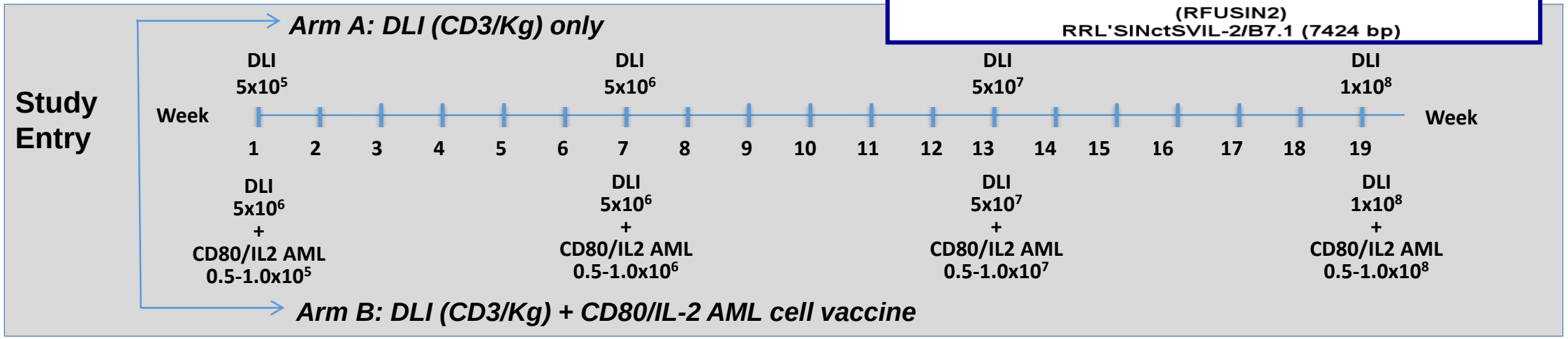
Phase-I Clinical trial

Relapsed AML following allogeneic HSCT

Pre-study entry conditions:

- Relapsed AML/MDS
- Post-allogeneic HSCT
- >50% donor CD3 chimerism at relapse
- <5% BM blast following cytoreductive chemotherapy

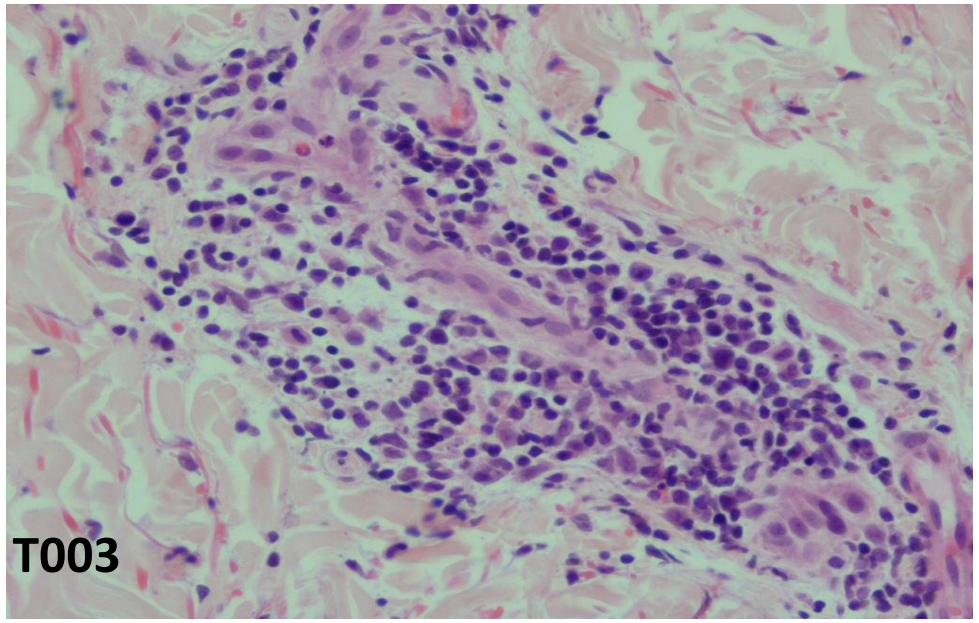




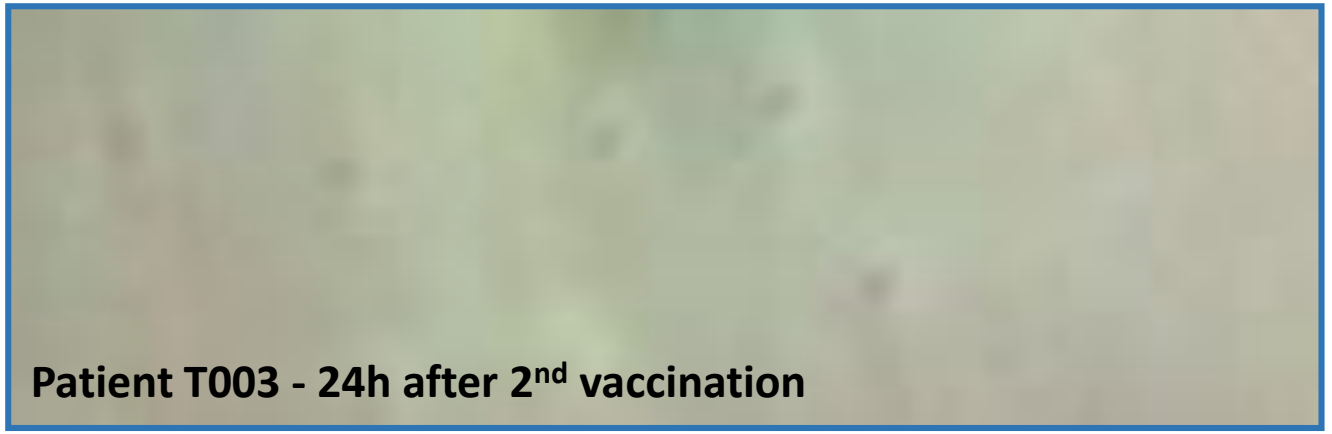
Vaccination: relapsed AML (post-HSCT), after the re-induction of remission

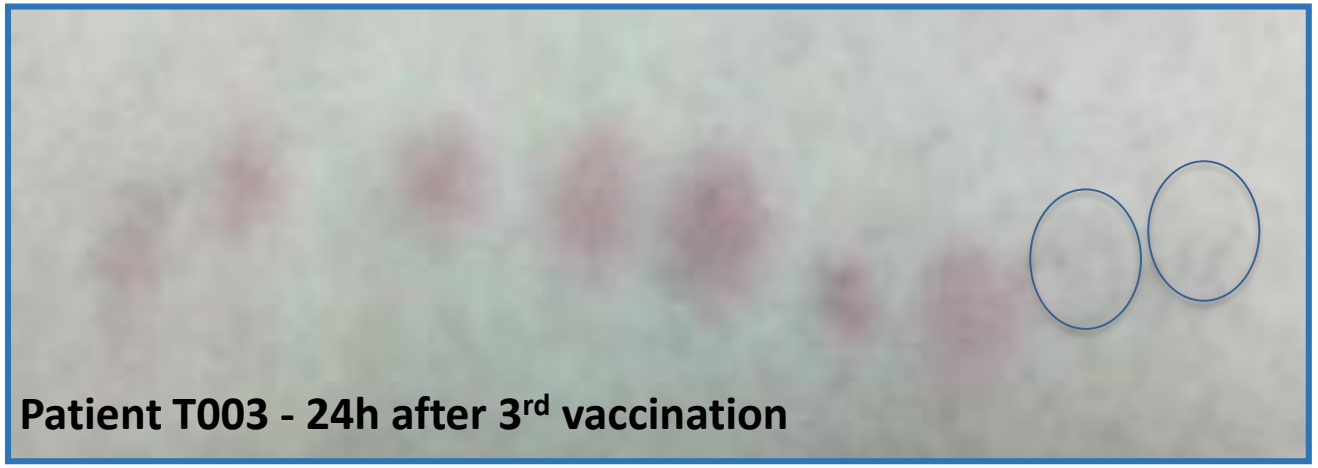
– therapeutic prophylaxis for the induction of deeper, longer lasting, remission

Delayed Type Hypersensitivity (DTH) following DLI + CD80/IL-2 AML cell vaccination



Skin biopsy 76h post 3rd injection





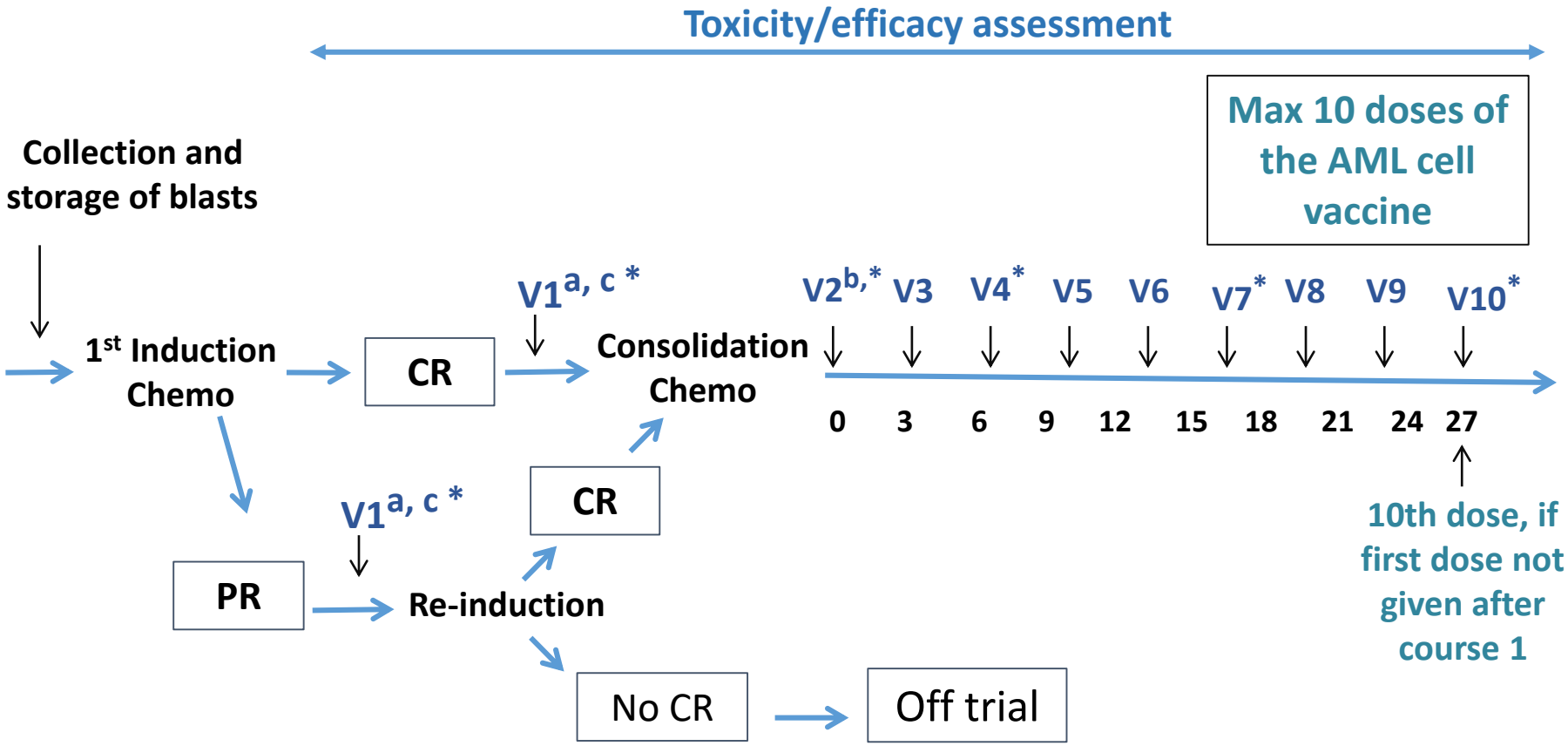
In complete cytogenetic and molecular CR since June 2011

7 Patients enrolled to date: - Confirmed feasibility
- No acute toxicities / adverse events
- Safety/efficacy studies completely satisfactory

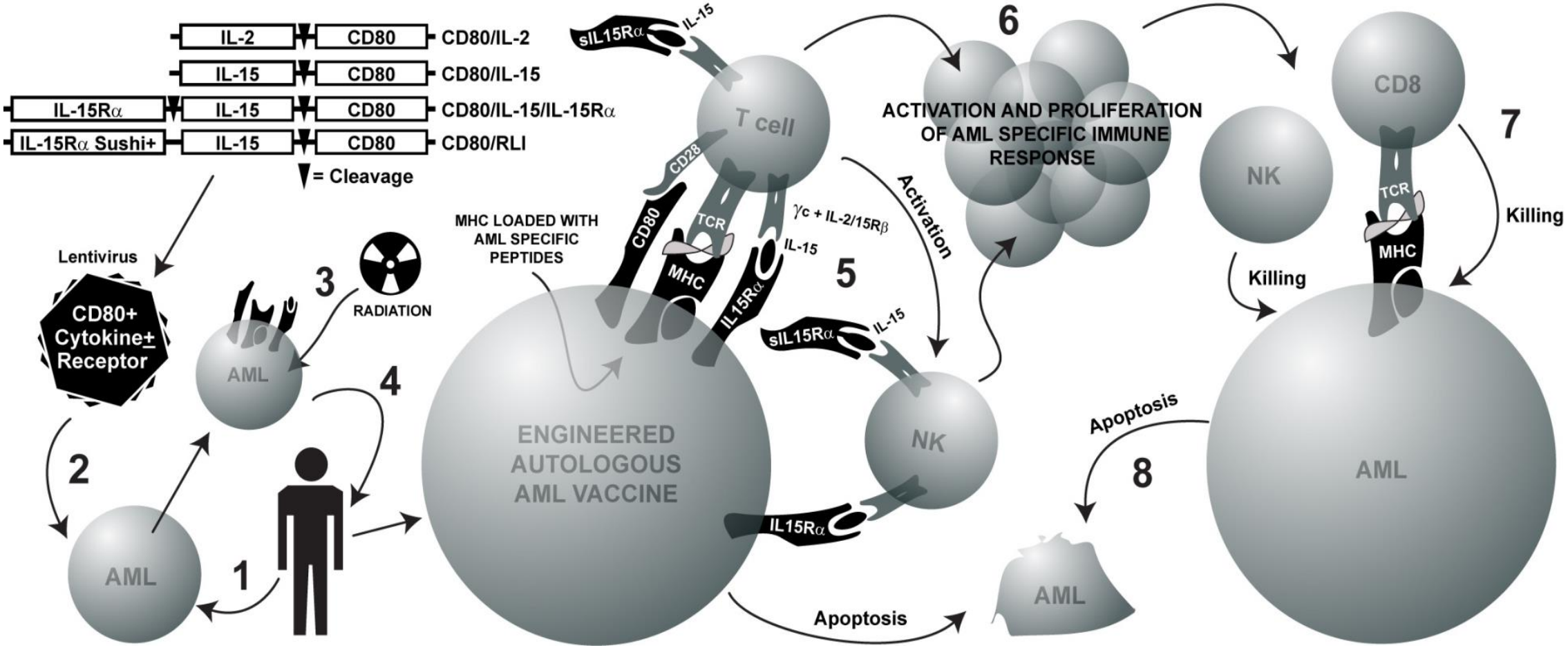
Phase-I Trial of Autologous AML Cell Vaccine, Following Standard Induction Chemotherapy

Target Population:

High risk AML patients unable to receive allogeneic stem cell transplantation



Pre-clinical studies with genetically engineered autologous AML vaccines

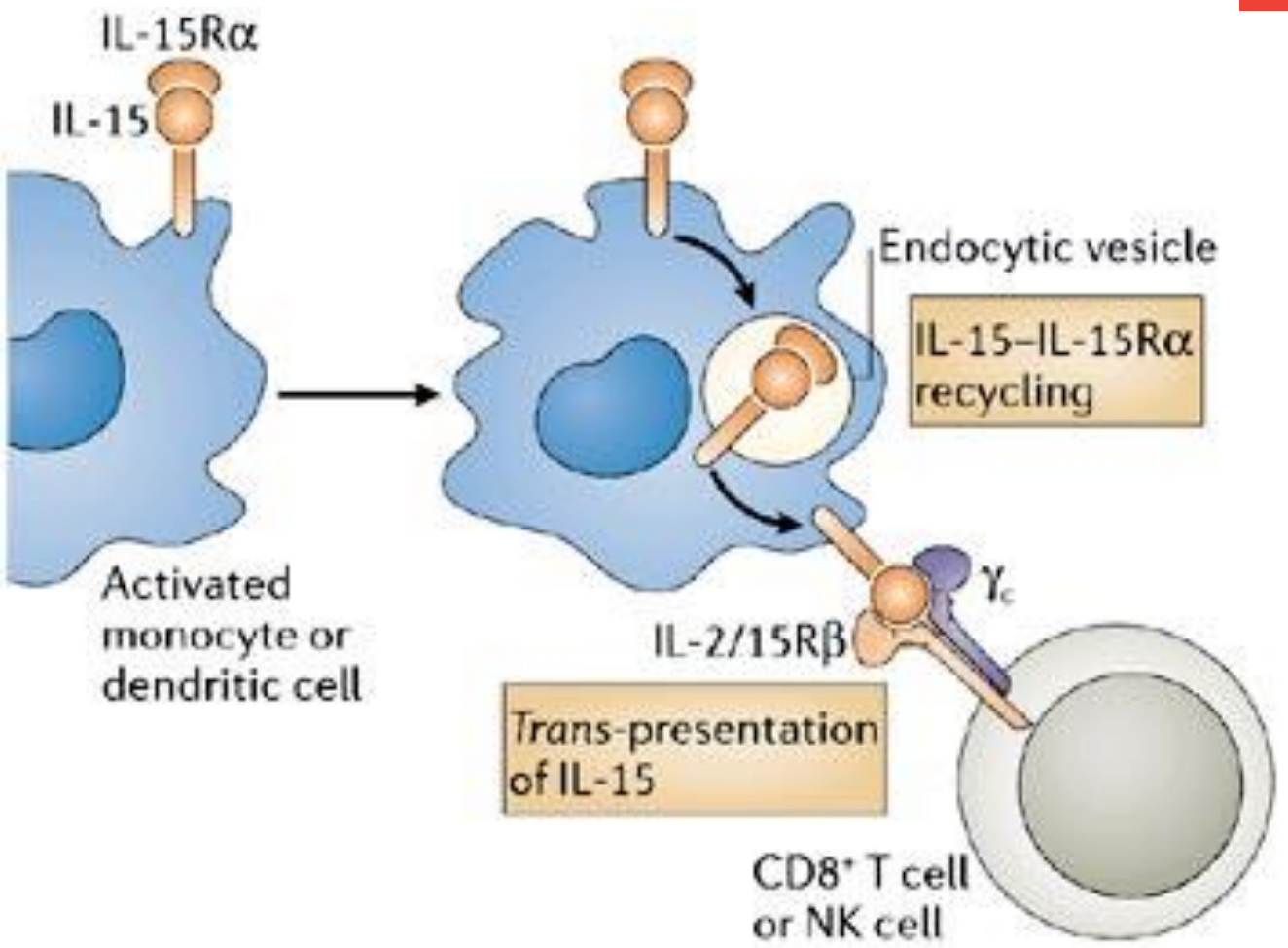


Shi Y *et al.*, 2018. Blood Advances. 2:3177-3192.

Trans-presentation of IL-15 by IL-15Rα

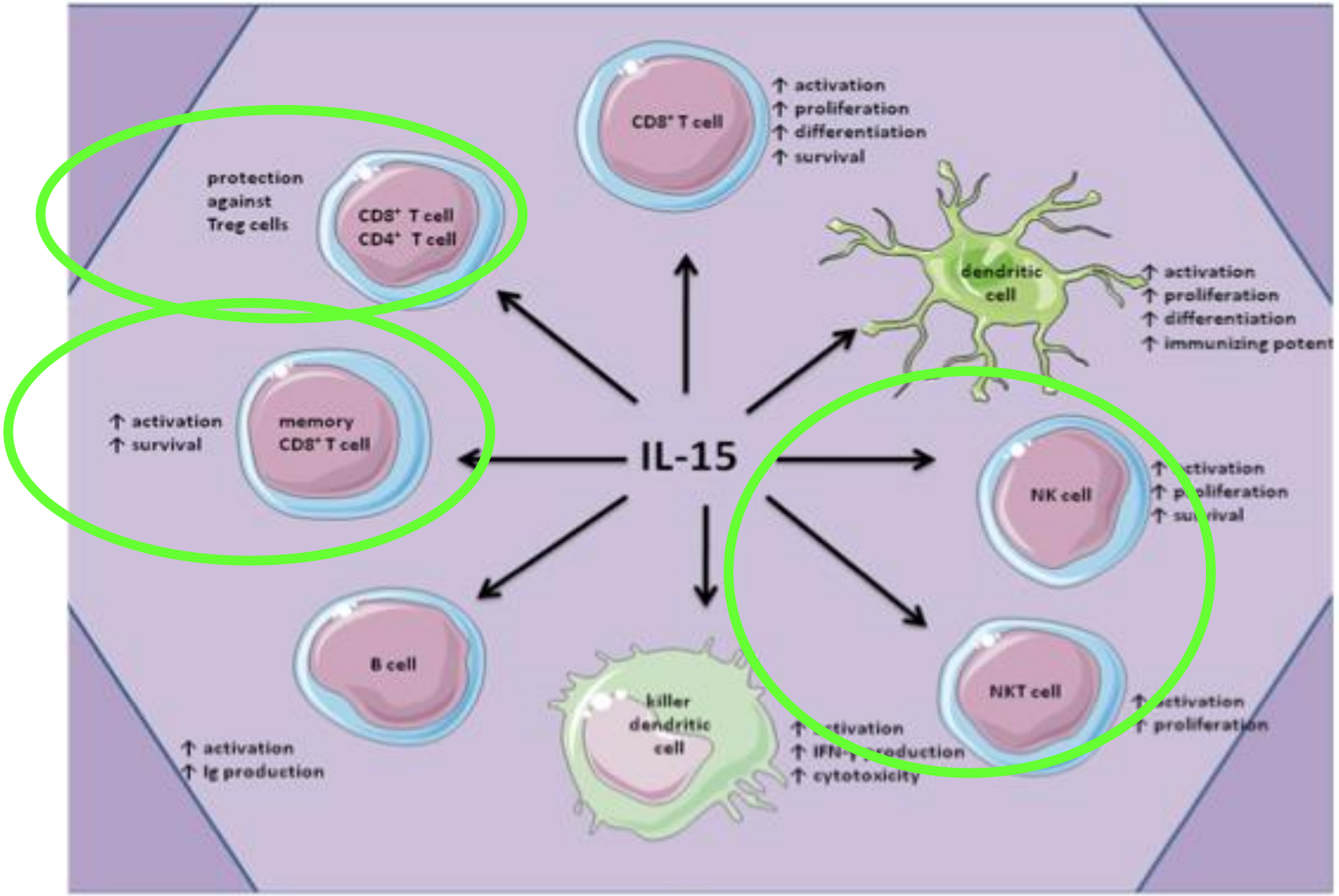
Higher and longer lasting efficacy

Co-expression of IL-15 and IL-15Ra markedly increases IL-15 stability and secretion.



Waldmann *Nature Reviews Immunology* 6, 595-601 (August 2006) | doi:10.1038/nri1901

IL-15 has important advantages as an immunostimulatory cytokine

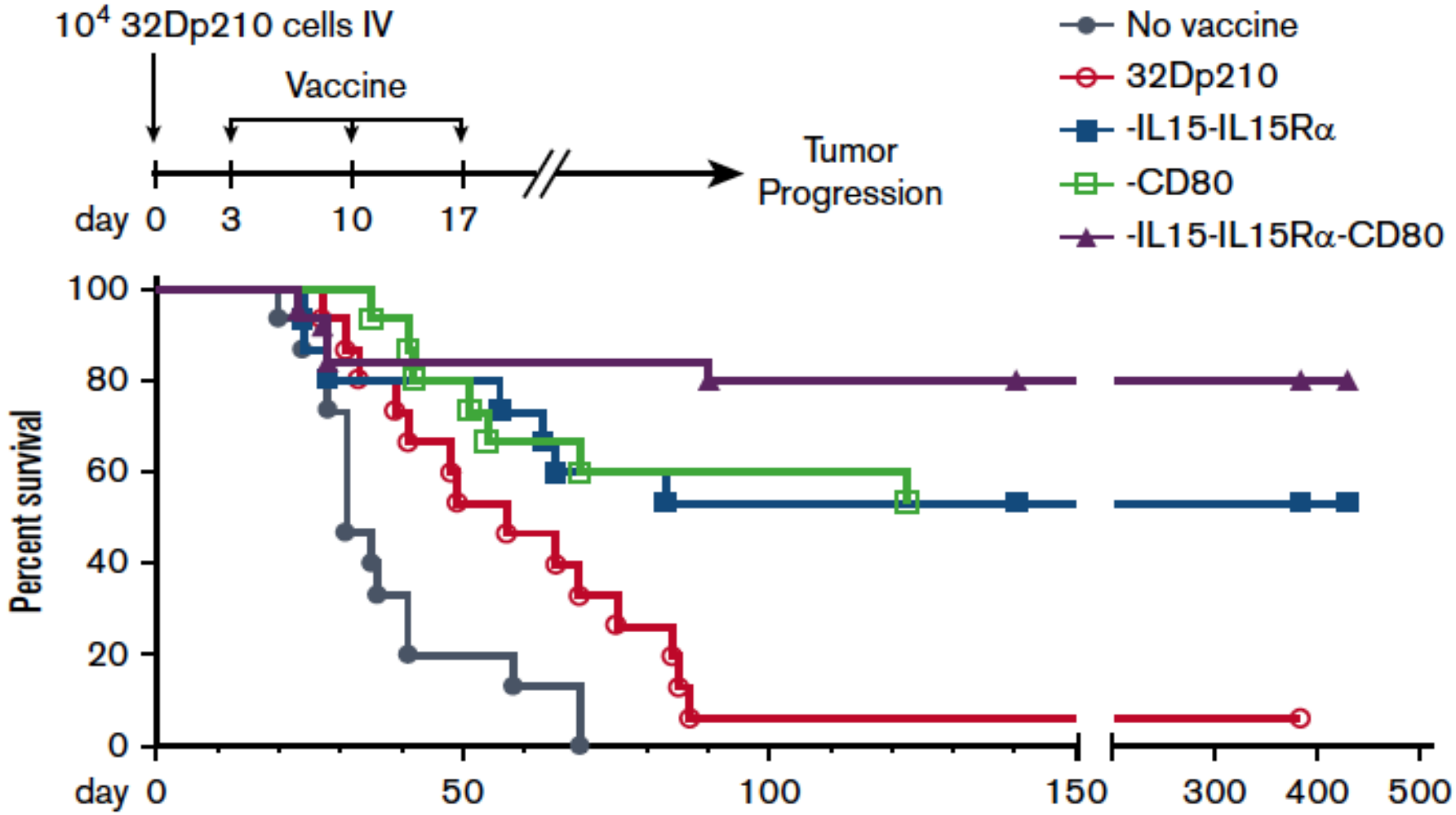


IL-15 vs IL-2

- In contrast to the effects of IL-2, IL-15 reverses CD8+ T-cell unresponsiveness to tumour-associated antigens, renders T effector cells resistant to suppressive regulatory T cells (Treg's),
- Participates in antiapoptotic signalling to effector T
- IL-15 stimulates more effective induction of antigen-specific cytotoxic lymphocytes
- More durable immunity through actions on memory T cells
- Important roles in natural killer (NK) and NK T-cell activation, proliferation, and survival.
- Less toxicity than IL-2 infusion, however it does cause neutropenia, fever, and other side effects.
- Finally, local expression of membrane-bound and secreted heterodimeric IL-15/IL-15Ra together with costimulation by CD80 may mimic the interactions of professional antigen-presenting cells with lymphocytes, required for triggering effective cell-mediated immune responses.

Vaccination with syngeneic cells expressing CD80/IL15/IL15Rα

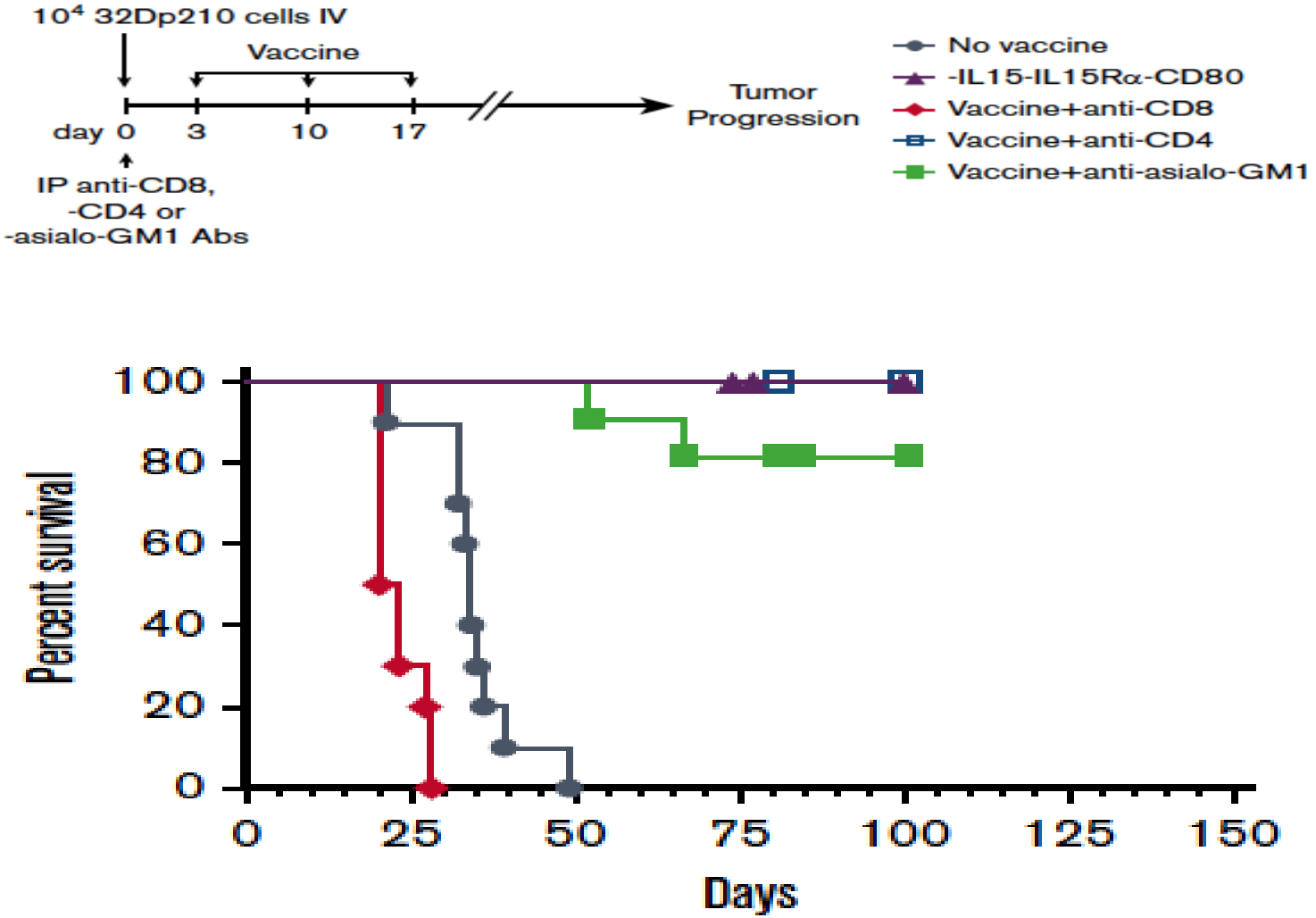
Efficient rejection of previously established AML by vaccination with syngenic cells expressing CD80/IL15/IL15Rα



Shi Y *et al.*, 2018. Blood Advances. 2:3177-3192.

TriLeukeVax AML cell vaccine efficacy requires the activation of CD8 T cells

In vivo antibody mediated depletion of CD8 T cells, but not CD4 or NK cells, abrogates the protection offered by TriLeukeVax



Shi Y *et al.*, 2018. Blood Advances. 2:3177-3192.

Lessons from Phase 1 clinical trial

(1) They can be reliably collected, cryopreserved, and engineered

(2)They can overcome tumour- related immunosuppression

(2)They can Stimulate leukaemia-specific cytolytic immune responses when administered either after remission/consolidation chemotherapy or in immunosuppressed patients early after hematopoietic stem cell transplantation.

The potent immune-stimulatory combination of heterodimeric IL-15/IL-15Ra and CD80 expression in autologous leukaemia cells provides a promising and universally applicable approach to generation of personalized leukaemia vaccine therapy that could improve progression-free survival.

Vaccination induced prevention of tumour recurrence

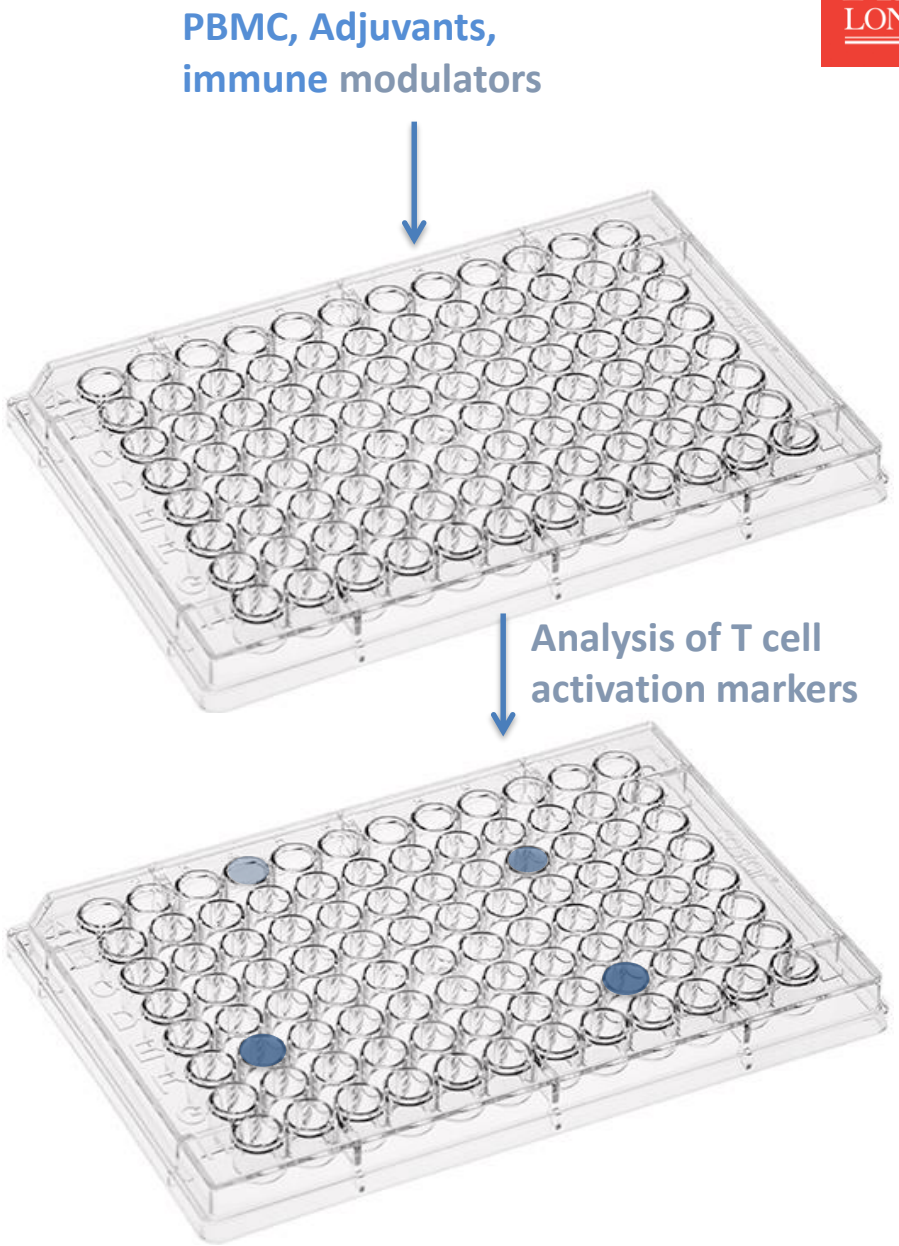
- by induction of antigen-specific cellular immunity

CASAC: Combined Adjuvants for Synergistic Activation of Cellular immunity

In vitro identification of combination of adjuvants / immune modulators synergistic activation cellular immunity

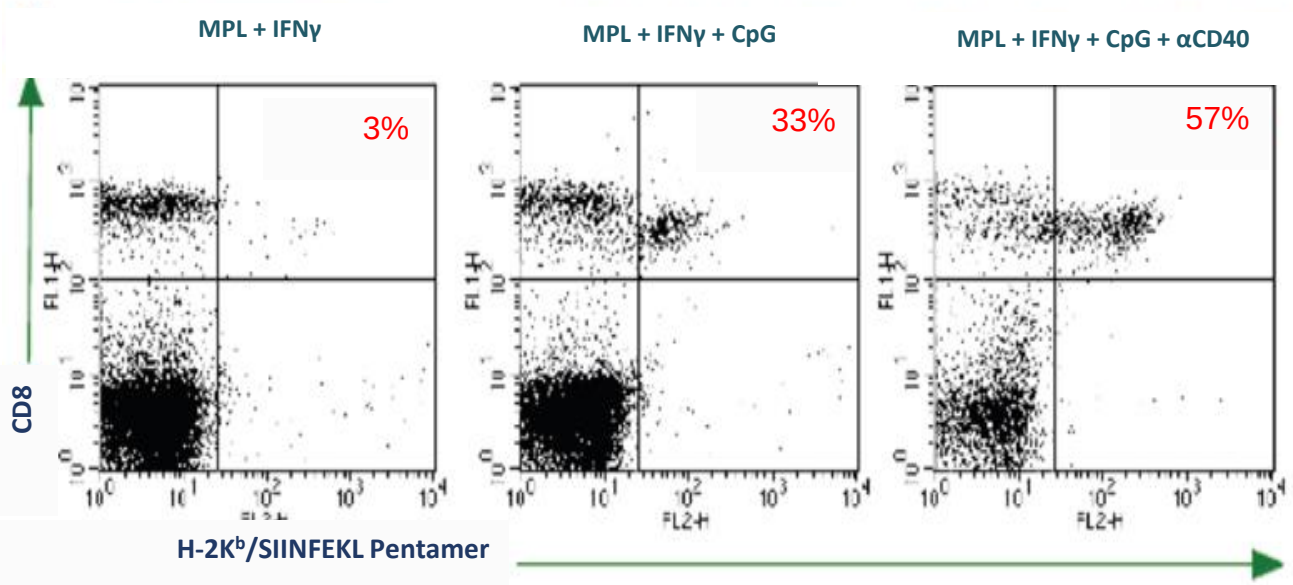
In vivo validation of combination of adjuvants able to induce antigen specific cytolytic activity

Identification of factors that individually or in combination stimulate appropriate T cell function (*e.g.* IFNγ , perforin, etc., expression and or target cell lysis)



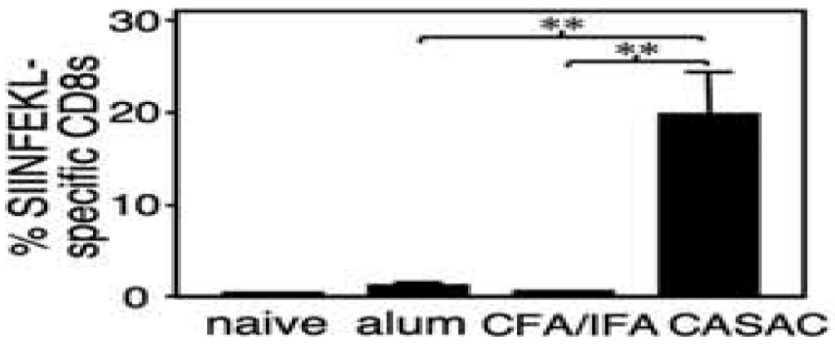
CASAC:

Combined Adjuvants for Synergistic Activation of Cellular immunity



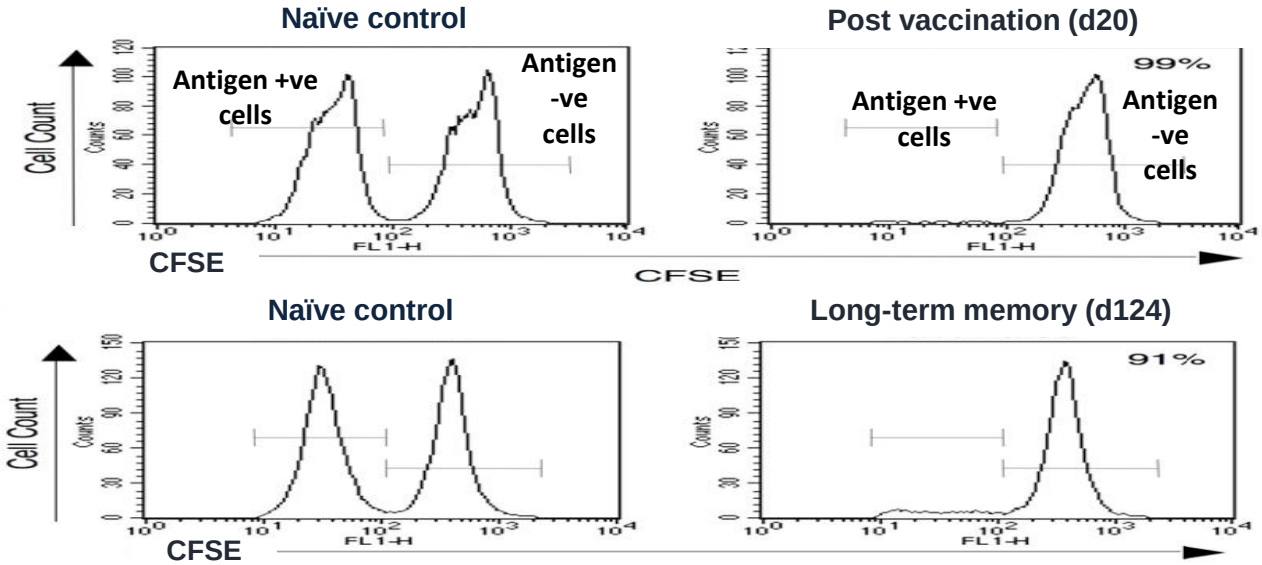
Extensive clonal expansion of CD8+ T cells with complementary TLR agonists + IFNγ + α-CD40

- Synergy between Myd88 and TRIF stimulation
- Long-term memory & *in vivo* Cytolytic activity



Wells et al. (2008). *J. Immunol.* 181(5): 3422-31.

In vivo CTL actisvity (lyis of antigen +ve cells)



New phase-I hTERT vaccination study
(started December 2015)

A first step in the clinical application of therapeutic CASAC cancer vaccines

Principal Investigators:

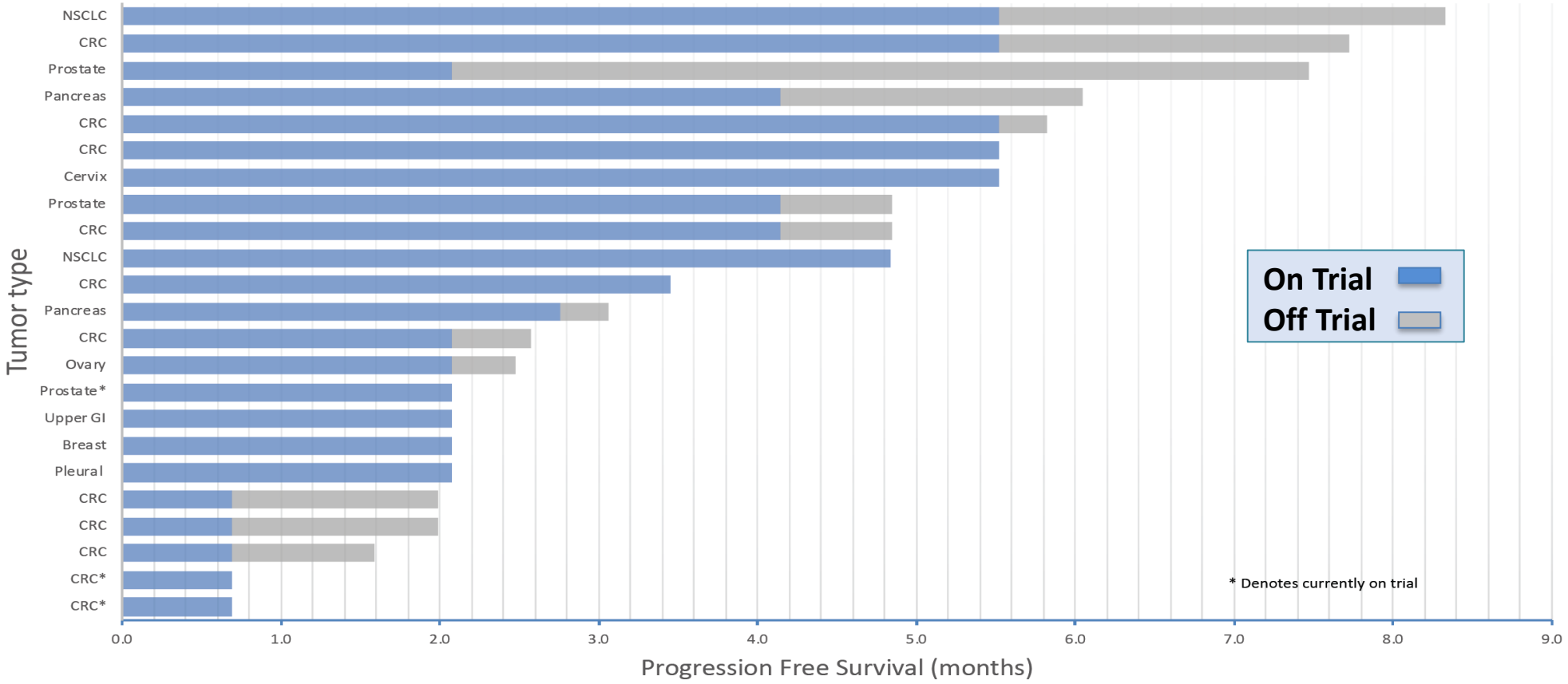
Alec Eremin
Farzin Farzaneh
Hardev Pandha
James Spicer

Synthetic hTERT peptide library:

- Multiple class-I epitopes (covers > 95% population)
- Multiple promiscuous class-II epitopes
- TLR7 Agonist (Imiquimod)
- Montanide (emulsion)
- Plus metronomic low dose cyclophosphamide – in order to reduce the number of regulatory T cells

A Phase-I clinical trial in 30 Patients with therapy resistant, progressive, metastatic disease

Progression free survival in VAPER Phase-I patients (interim data)



To date: 25 Patients with therapy resistant, progressive, metastatic disease:

- Disease stasis (Progression Free Survival for greater than 5 months in 8 patients (~ 30%), 5 with > 6 months pfs

Regeneration link with Cancer

Small RNAs (miRNA)

as

Markers and Therapeutic tools in Cancer

Regeneration is a developmental process that involves growth, morphogenesis and differentiation

Types of Regeneration

1) Physiological Regeneration

Replacement of R.B.C's

Replacement of Epidermal Cells of the Skin

2) Autotomy

Crabs break off their leg on approaching of the enemy
Holothurians throw off their internal viscera
Starfish breaks off an arm

3) Repair

***Regeneration**

Regeneration of limbs in salamanders
Regeneration of lost tail in lizard cells

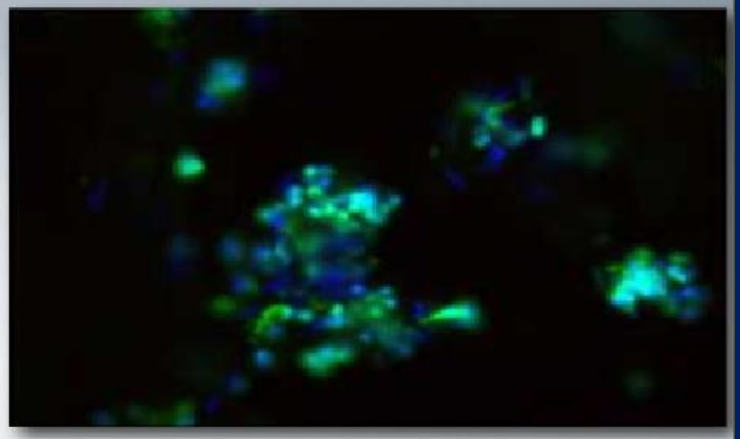
*** Reconstitution**

Healing of wound
Replacement of damaged cells

"A processes that allows an organism to regain the function of an organ or structure damaged by injury or disease"

BUT....

Regeneration ⟷ **Repair** ⟷ **Reconstitution**



KING'S HEALTH PARTNERS

MRC Centre for Transplantation

REGENERATIVE CAPACITY AND EVOLUTION

Hydra

1/1000 of body regenerate the whole

Planarians

Small fragments of the body can give rise to a whole animal.

Drosophila

Can regenerate limbs

Axolotl

Zebrafish

Fishes: Lamprey can regenerate its lost tail. Some fishes have the ability to regenerate parts of its fins.
Amphibians: salamanders, newts and their axolotl larvae. They can regenerate limbs, tail, external gills, jaws, parts of eye like lens and retina. Tail and limb regeneration is found in the larval stages of frogs and toads.
Reptiles: Lizards exhibit autotomy

Rodent

Primate

Mammals: Regeneration is restricted to tissues only.
The liver has the maximum capacity of regeneration.

Spong

The entire body can be reconstructed from isolated body cells

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

REGENERATIVE CAPACITY AND EVOLUTION

Hydra

Planarians

Drosophila

Axolotl

Zebrafish

Rodent

Primate

regenerative potential

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

REGENERATIVE CAPACITY AND EVOLUTION

Hydra

Planarians

Drosophila

Axolotl

Zebrafish

Rodent

Primate

longevity

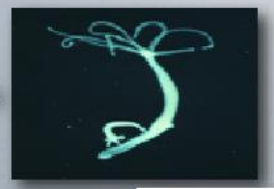
regenerative potential

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

REGENERATIVE CAPACITY AND EVOLUTION

Hydra



Planarians

Drosophila

Axolotl

Zebrafish

Rodent

Primate

longevity

regenerative potential

cancer risk

KING'S HEALTH PARTNERS

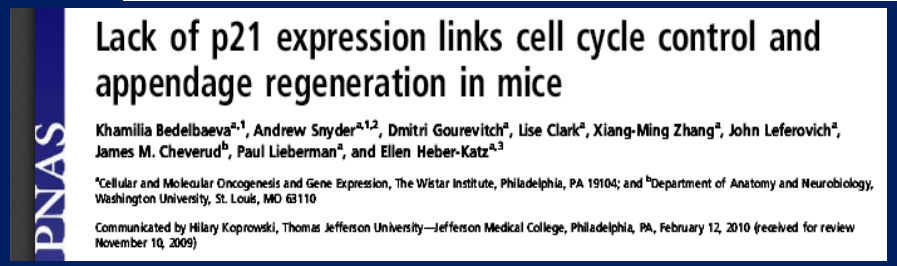
MRC Centre for Transplantation

Am J Physiol Gastrointest Liver Physiol 304: G26–G37, 2013.
First published October 25, 2012; doi:10.1152/ajpgi.00064.2012.

Hepatocyte-specific deletion of hepatocyte nuclear factor-4α in adult mice results in increased hepatocyte proliferation

Chad Walesky,[1] Sumedha Gunewardena,[2] Ernest F. Terwilliger,[3] Genea Edwards,[1] Prachi Borude,[1] and Udayan Apte[1]

[1]Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas; [2]Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas; and [3]Division of Experimental Medicine, Beth Israel Deaconess Medical Center, and Harvard Medical School, Boston, Massachusetts

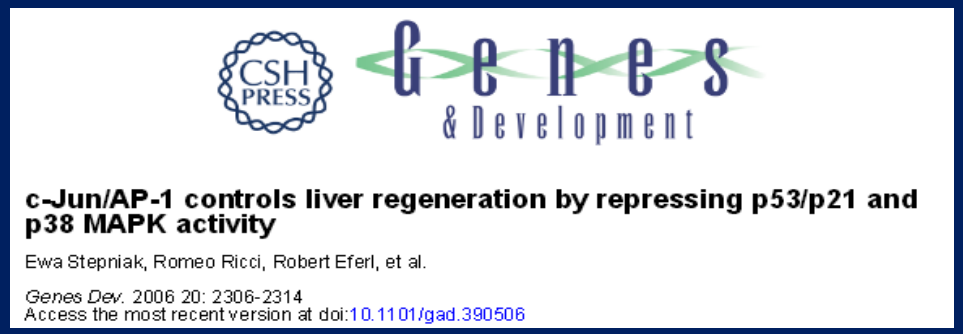


Lack of p21 expression links cell cycle control and appendage regeneration in mice

Khamilia Bedelbaeva[a,1], Andrew Snyder[a,1,2], Dmitri Gourevitch[a], Lise Clark[a], Xiang-Ming Zhang[a], John Leferovich[a], James M. Cheverud[b], Paul Lieberman[a], and Ellen Heber-Katz[a,3]

[a]Cellular and Molecular Oncogenesis and Gene Expression, The Wistar Institute, Philadelphia, PA 19104; and [b]Department of Anatomy and Neurobiology, Washington University, St. Louis, MO 63110

Communicated by Hilary Koprowski, Thomas Jefferson University—Jefferson Medical College, Philadelphia, PA, February 12, 2010 (received for review November 10, 2009)



CSH PRESS Genes & Development

c-Jun/AP-1 controls liver regeneration by repressing p53/p21 and p38 MAPK activity

Ewa Stepniak, Romeo Ricci, Robert Eferl, et al.

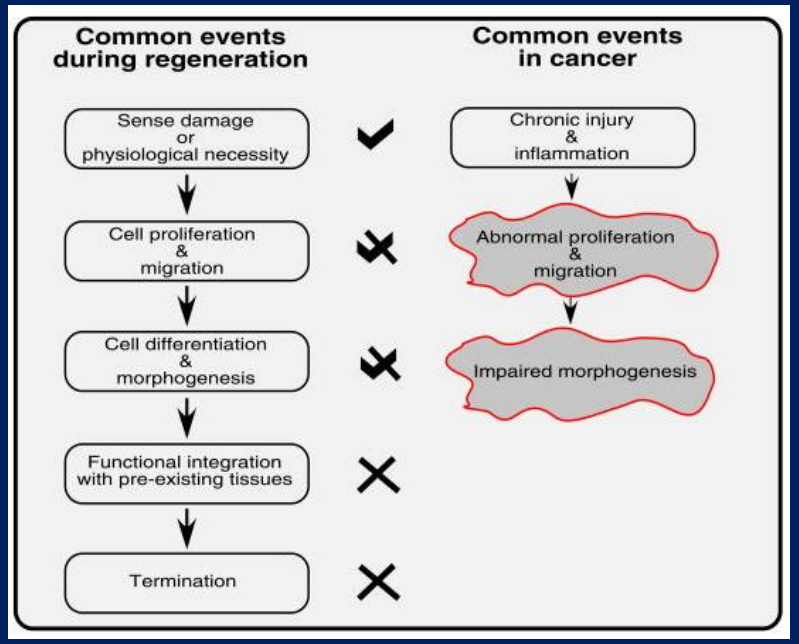
Genes Dev. 2006 20: 2306-2314
Access the most recent version at doi:10.1101/gad.390506

Published May 31, 2010

JEM Artic

Tob1 is a constitutively expressed repressor of liver regeneration

Karen J. Ho,[1,3,4] Nhue L. Do,[1,4] Hasan H. Otu,[2] Martin J. Dib,[1,3] Xianghui Ren,[2] Keiichi Enjyoji,[2] Simon C. Robson,[2,3] Ernest F. Terwilliger,[2] and Seth J. Karp[1,3]

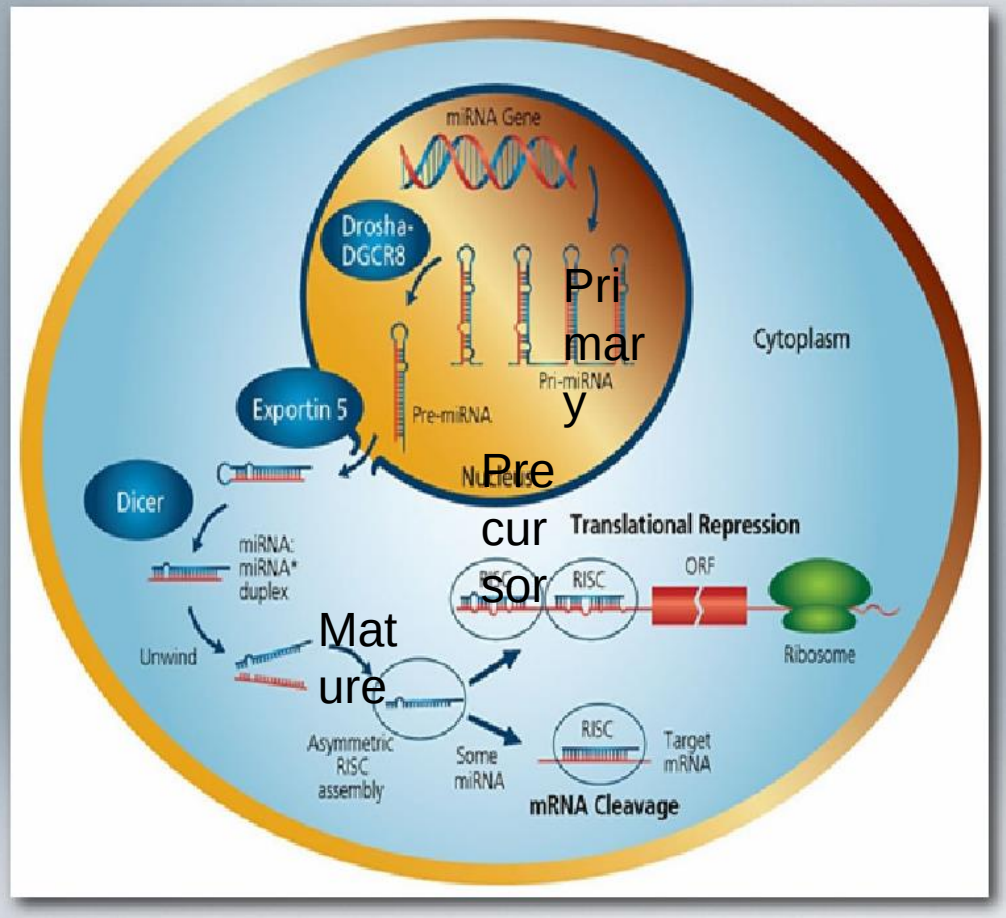


Regenerative events and their corollaries in cancer. Importantly, the process of regeneration can be repeated without causing malignant transformation, while in cancer the regenerative process is incomplete such that chronic injury and inflammation leads to continuous proliferation. This suggests that characterizing signals at later stages of regeneration (especially those involved in termination) may help identify candidates able to stop abnormal proliferative responses to chronic injury.

Regeneration: Regulated cell proliferation

Cancer : Dysregulated cell proliferation

MIRNA



Pri mar y

Pre cur sor

Mat ure

Functions:

Regulate development

Protect agianst viruses

Maintain genome stability

Epigenetic global regulation

Tools:

* Specific gene suppression
• Gene Therapy

LNAs : Locked Nucleic Acids
AMOs: anti-miRNA oligos
Antagomirs: (chemical stability)
miRNA replacement therapy
Enoxacin: small molecule drug, miRNA sponge

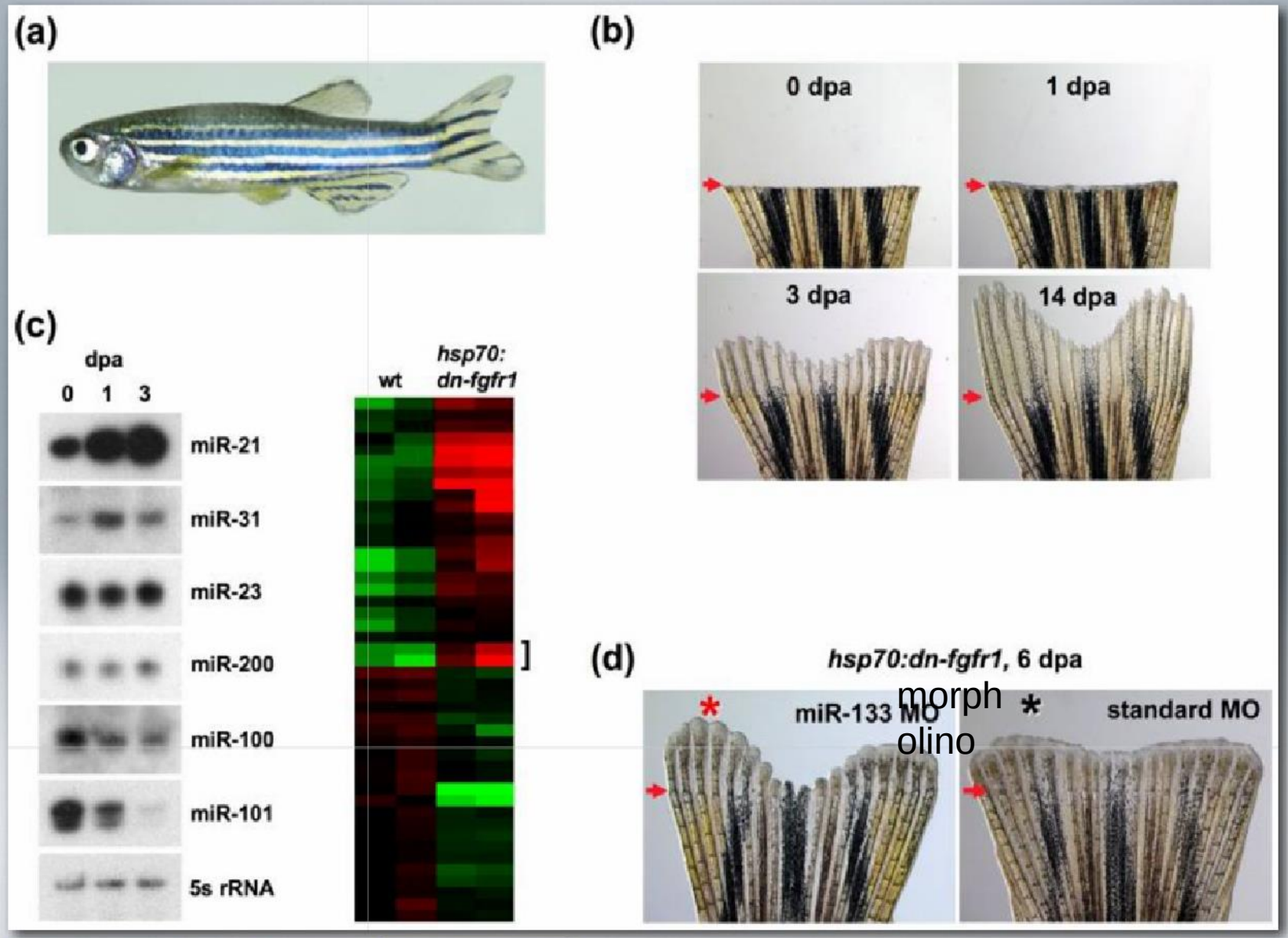
miRNAs are processed sequentially from primary miRNA (pri-miRNA) precursor transcripts, and regulate gene expression at the post-transcriptional level.

miRNA = rapid large-scale specific gene regulation

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

MIRNA AND REGENERATION



KING'S HEALTH PARTNERS

MRC Centre for Transplantation

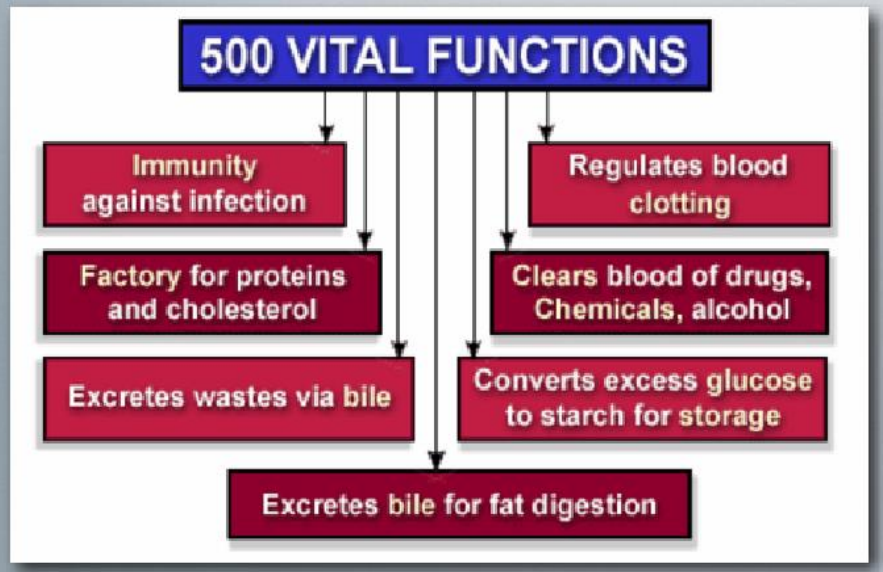
Yin & Poss Genes & Dev 2008

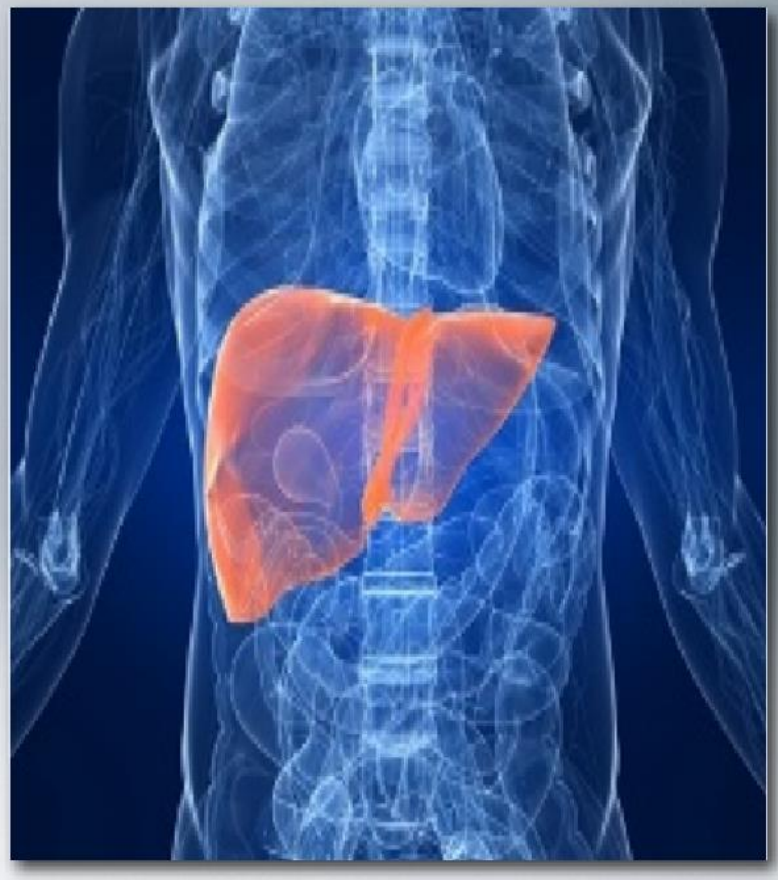
WHY THE LIVER?

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

HIGHER VERTEBRATE LIVER REGENERATION





Maintain vital functions in life

Recover function after major insults

Surgery/Transplantation/Cell Rx

'Silent damage'

Chronic diseases/Cirrhosis

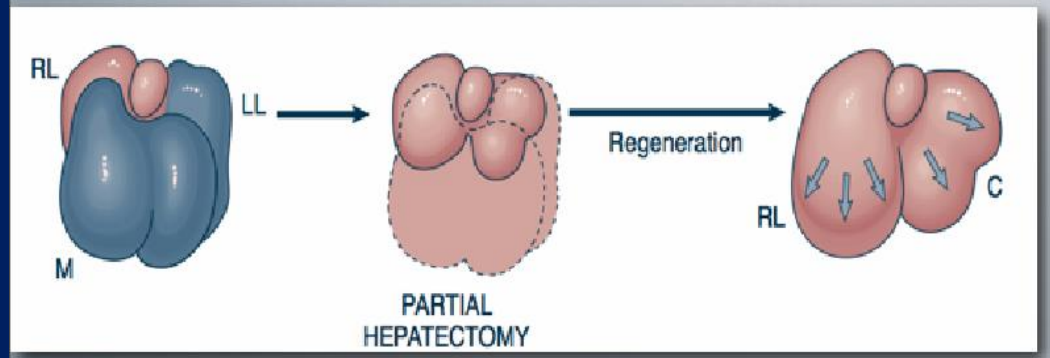
Cancer risk

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

HIGHER VERTEBRATE LIVER REGENERATION

Rodent partial-hepatectomy model



'Quick'

'Clean/surgical'

'mouse/rat not man'

'Inbred'

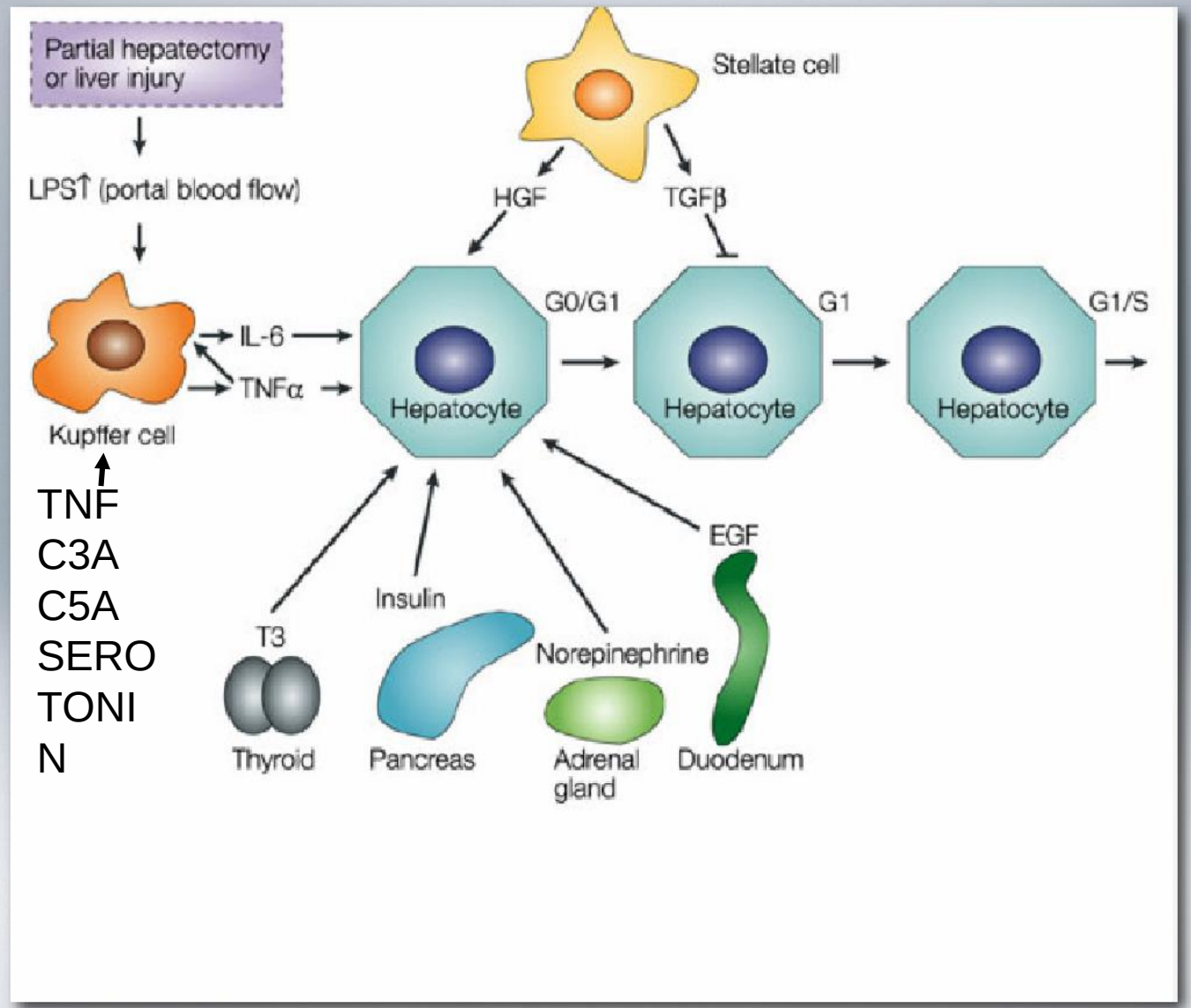
No disease

Human liver regeneration

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

REGENERATION: PARTIAL HEPATECTOMY

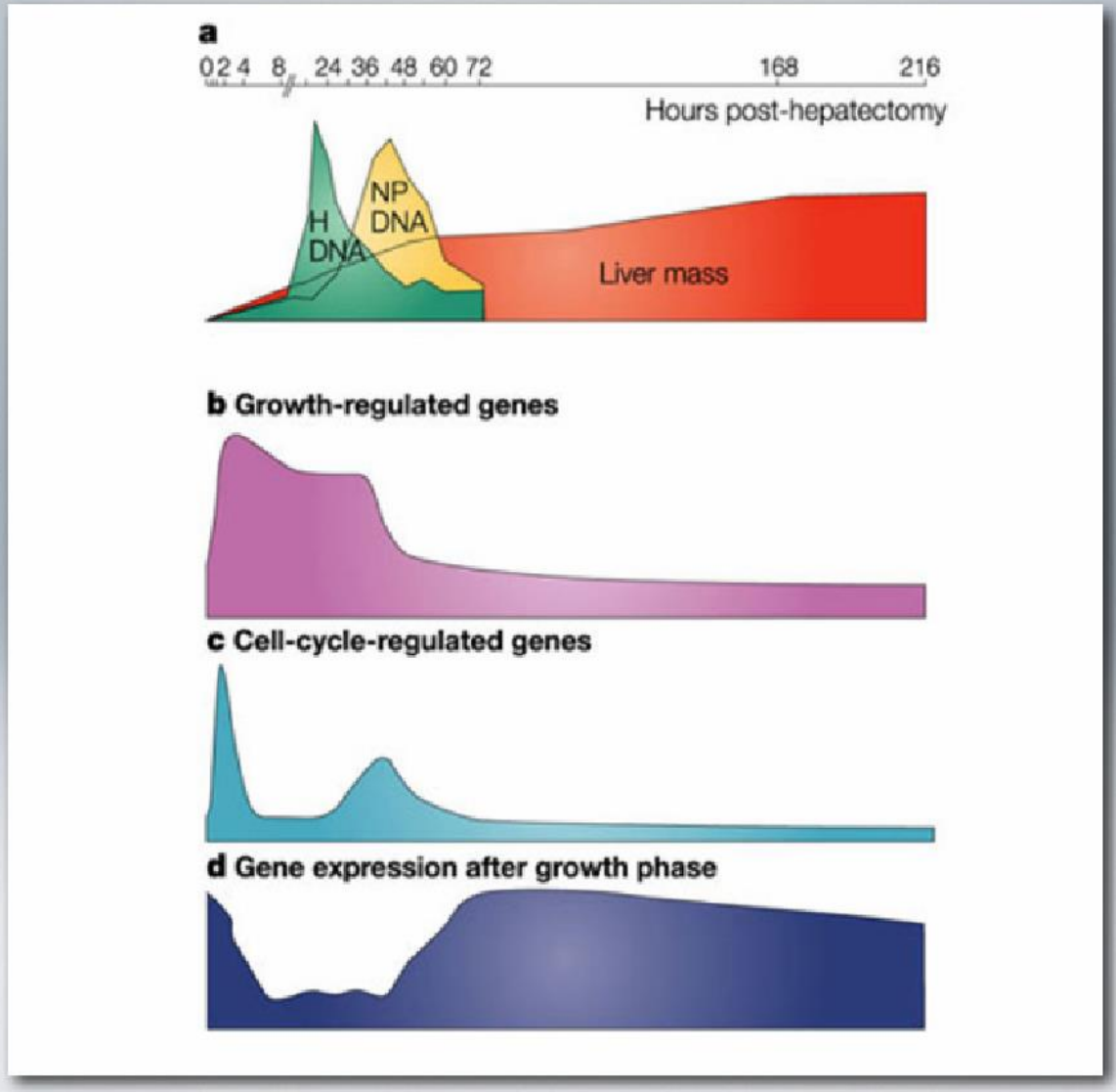


TNF
C3A
C5A
SERO
TONI
N

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

REGENERATION: PARTIAL HEPATECTOMY



KING'S HEALTH PARTNERS

MRC Centre for Transplantation

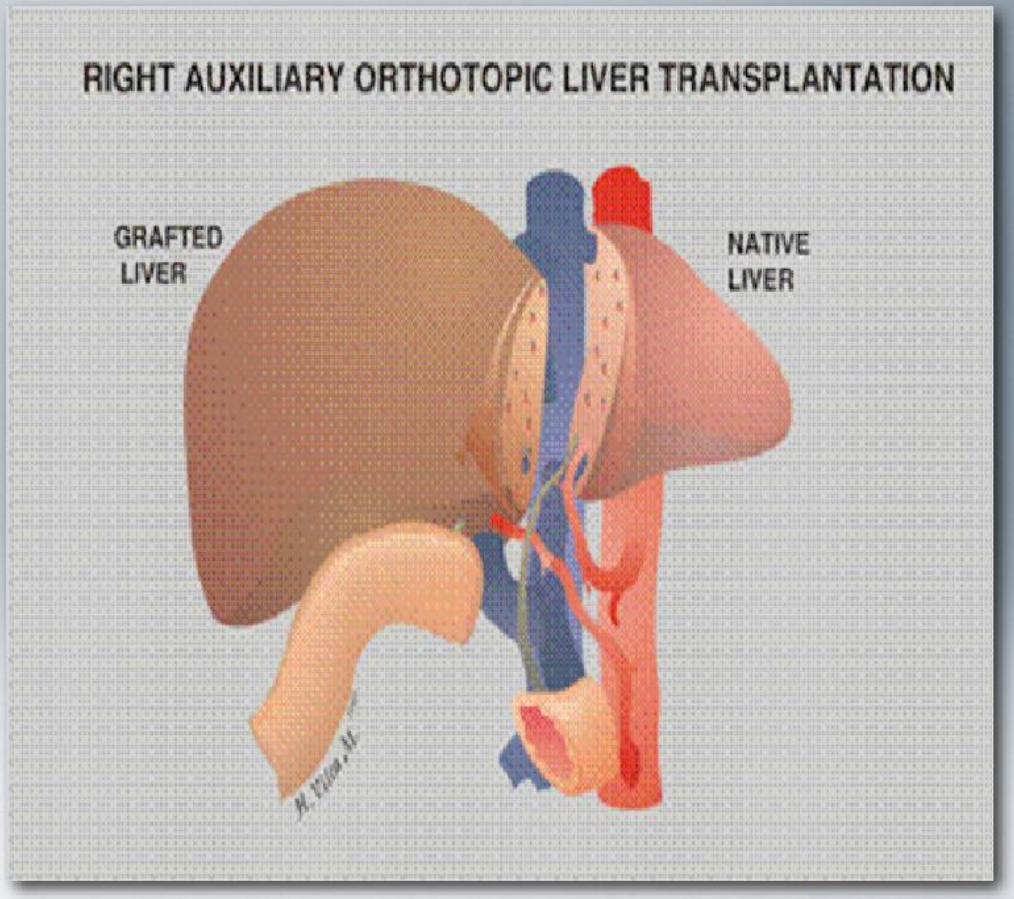
AUXILIARY TRANSPLANTATION

Acute liver failure

Strict selection criteria

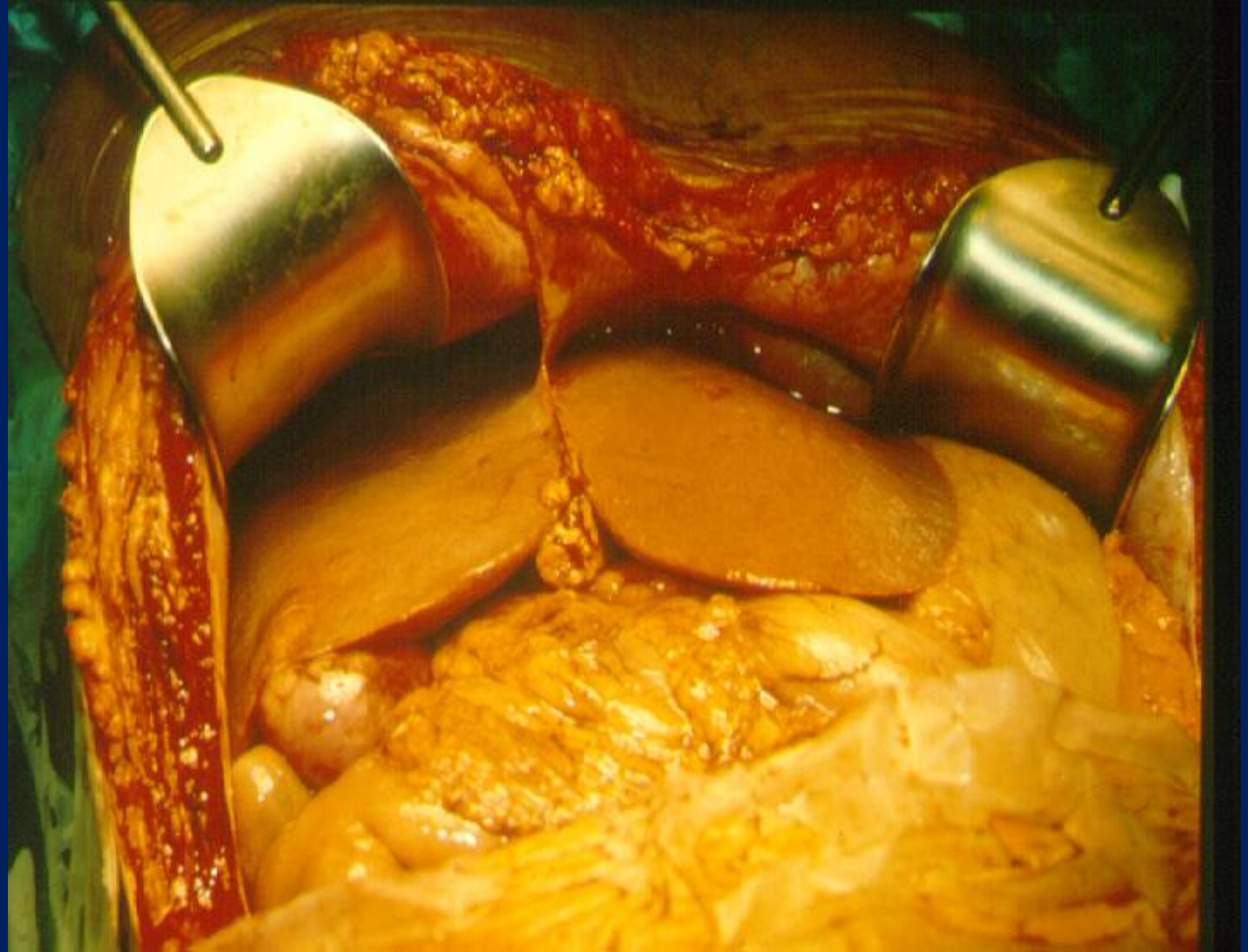
Conventional IS

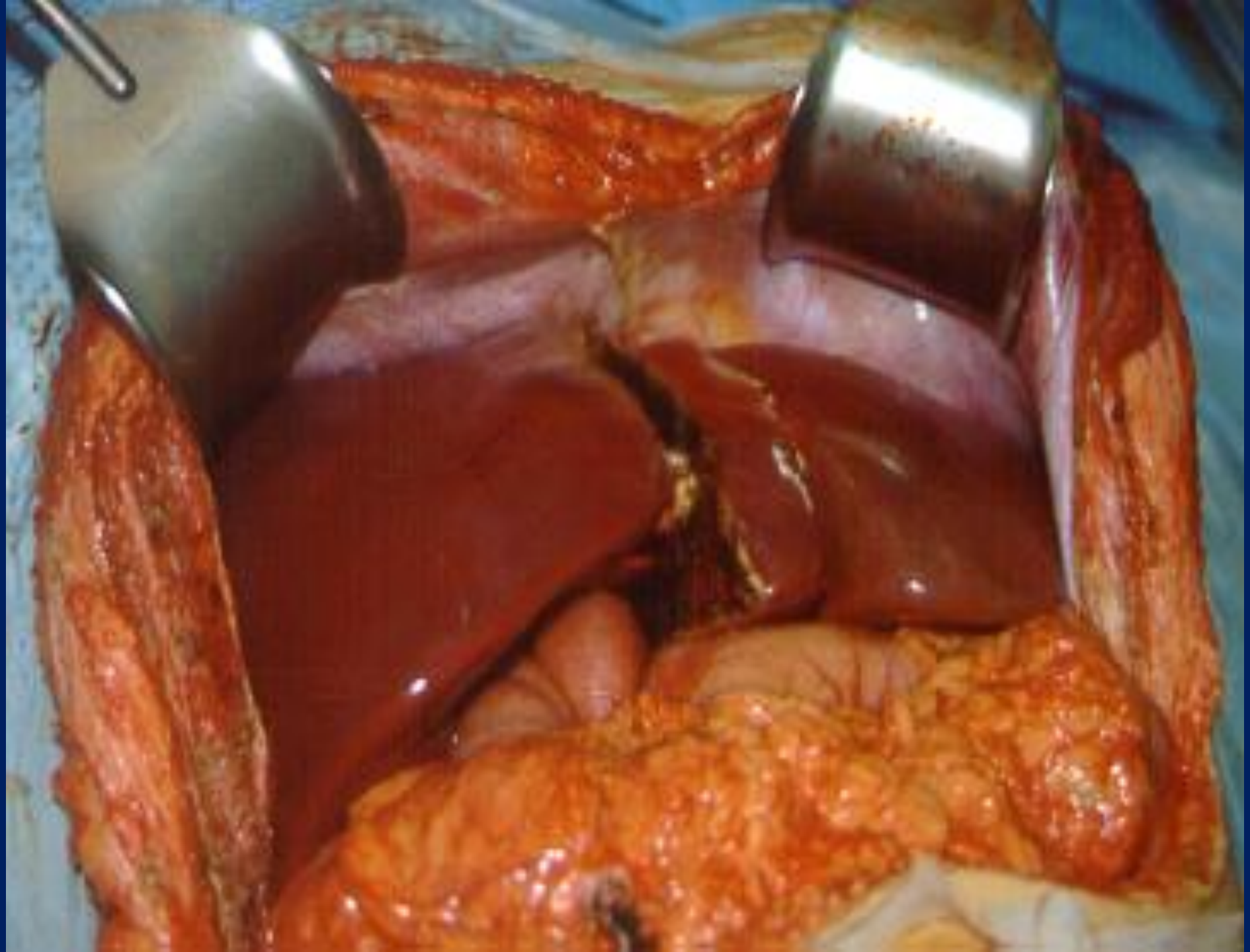
> 60% weaned off IS

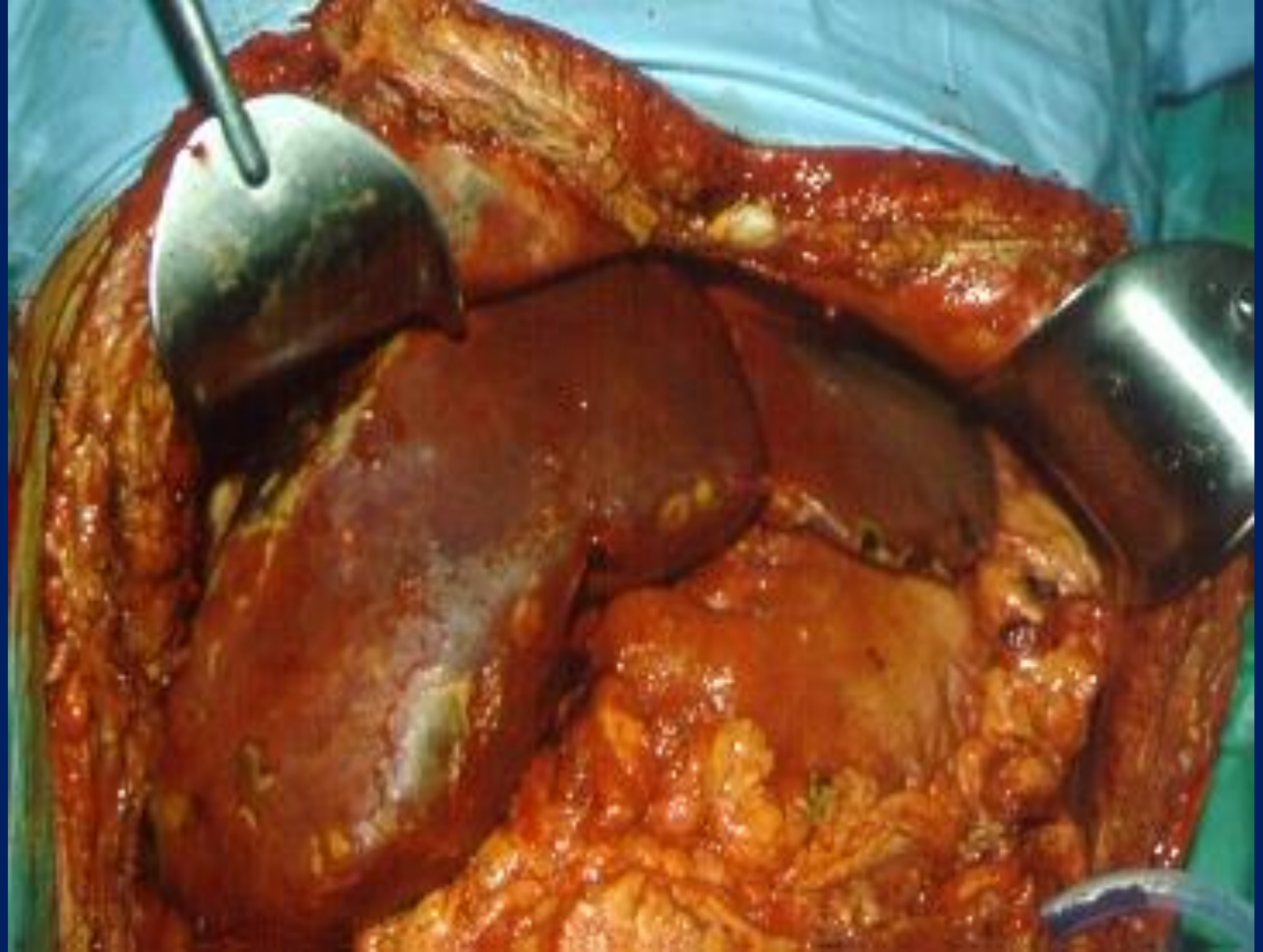


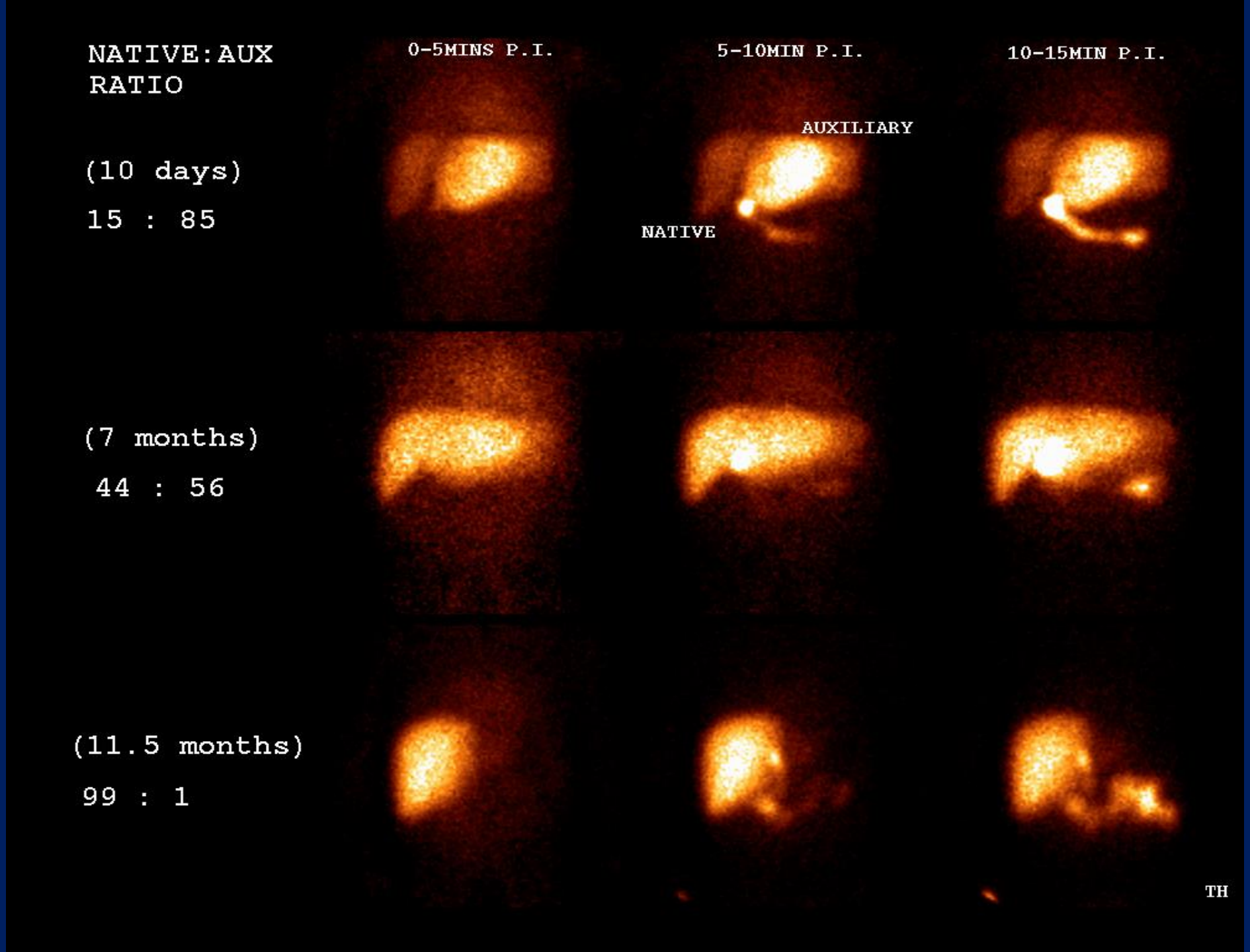
KING'S HEALTH PARTNERS

MRC Centre for Transplantation

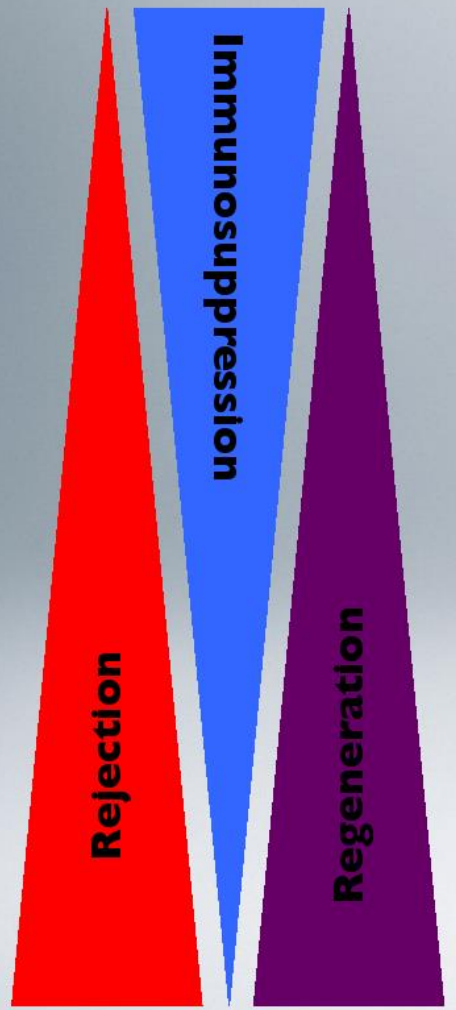


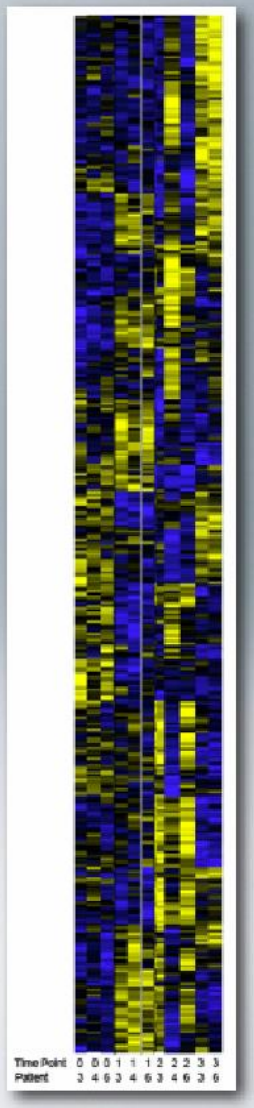





NATIVE:AUX
RATIO
(10 days)
15 : 85
(7 months)
44 : 56
(11.5 months)
99 : 1
0-5MINS P.I.
5-10MIN P.I.
10-15MIN P.I.
AUXILIARY
NATIVE
TH

AUXILIARY LIVER TRANSPLANTATION: SIMULTANEOUS CHARACTERISATION OF LIVER REGENERATION AND REJECTION





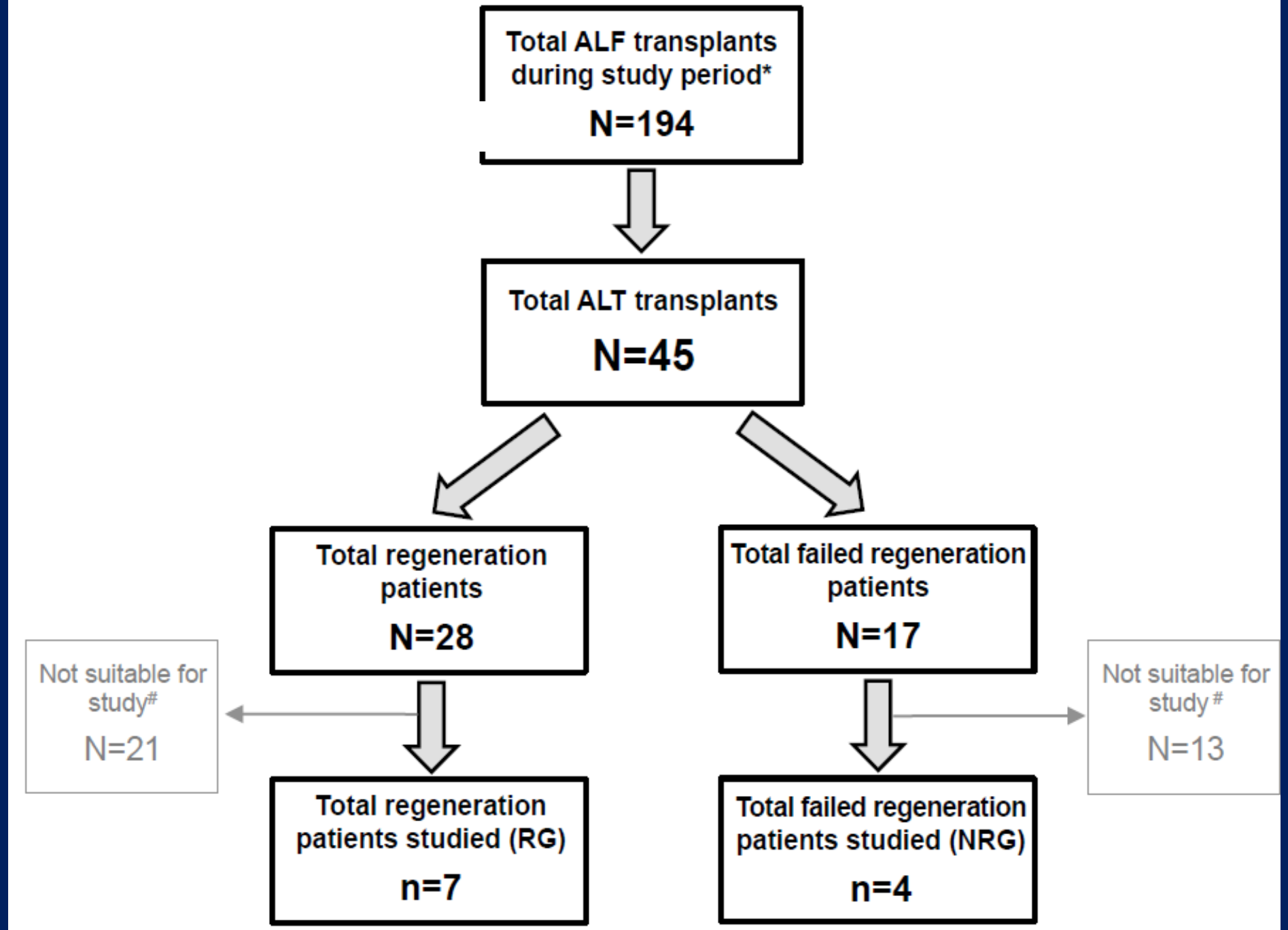
KING'S HEALTH PARTNERS

MRC Centre for Transplantation

AUXILIARY LIVER TRANSPLANTATION FOR ACUTE LIVER FAILURE

Choice of recipient

- Satisfy current transplant criteria
- Age less than 40 years
- Potential for regeneration – age, aetiology, fibrosis
- Aetiology of liver failure – toxic liver syndrome
- Clinical status – cardiovascular and neurological
- Quality of donor liver


Total ALF transplants during study period*
N=194
Total ALT transplants
N=45
Total regeneration patients
N=28
Total failed regeneration patients
N=17
Not suitable for study#
N=21
Not suitable for study #
N=13
Total regeneration patients studied (RG)
n=7
Total failed regeneration patients studied (NRG)
n=4

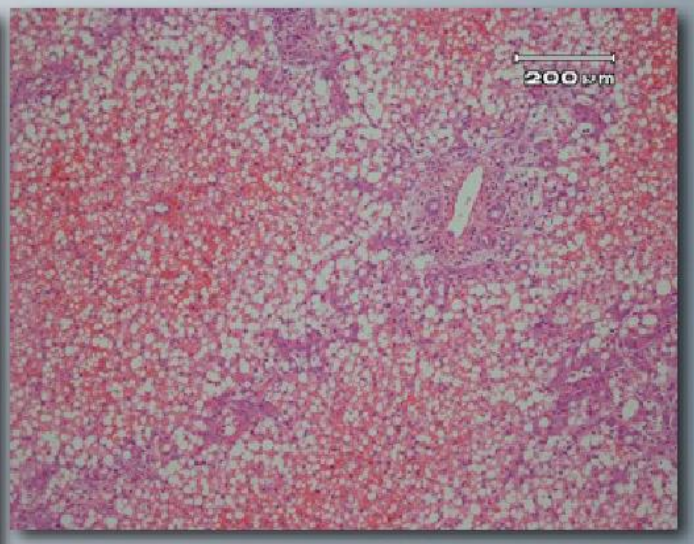
AUXILIARY LIVER TRANSPLANTATION: SIMULTANEOUS CHARACTERISATION OF LIVER REGENERATION AND REJECTION

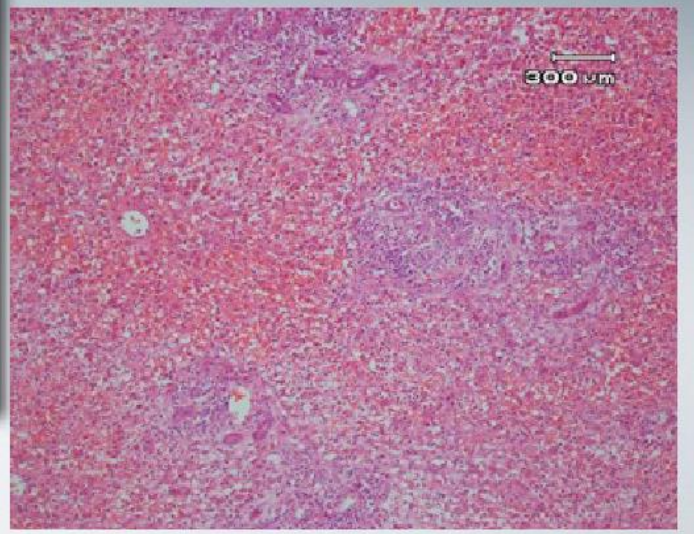
Regenerator patients R1-7

	Sex	Age	Indication	I/S	Withdrawal date	Rejection (graft)?	Regen.?
R1	M	17	Drug induced	FK/MMF	Weaning	Y-Day 8	Y
R2	F	4	Mushroom poison	FK	After 8 years	Y-Day 21	Y
R3	F	15	POD	FK	After 6 months	Y-Day 246	Y
R4	F	32	POD	FK	After 9 months	Y-Day 146	Y
R5	M	14	Seronegative	Cya	After 21 months	Y-Day 0	Y
R6	M	2.3	Seronegative	Cya/MMF	After 13 months	Y-Day 7	Y
R7	M	8	Seronegative /aplastic anaemia	FK/Cya	After 17 months	Y-Day 105	Y

Non-Regenerator patients NR1-4

	Sex	Age	Indication	I/S	Withdrawal date	Rejection (graft)?	Regen.?
NR1	M	32	Seronegative	Cya/FK	N/A	?	N
NR2	F	17	Drug induced	FK/MMF/ Basiliximab	N/A	?	N*
NR3	F	29	Drug induced (anti- TB)	FK	N/A	?	N
NR4	M	12	Seronegative	FK	N/A	?	N

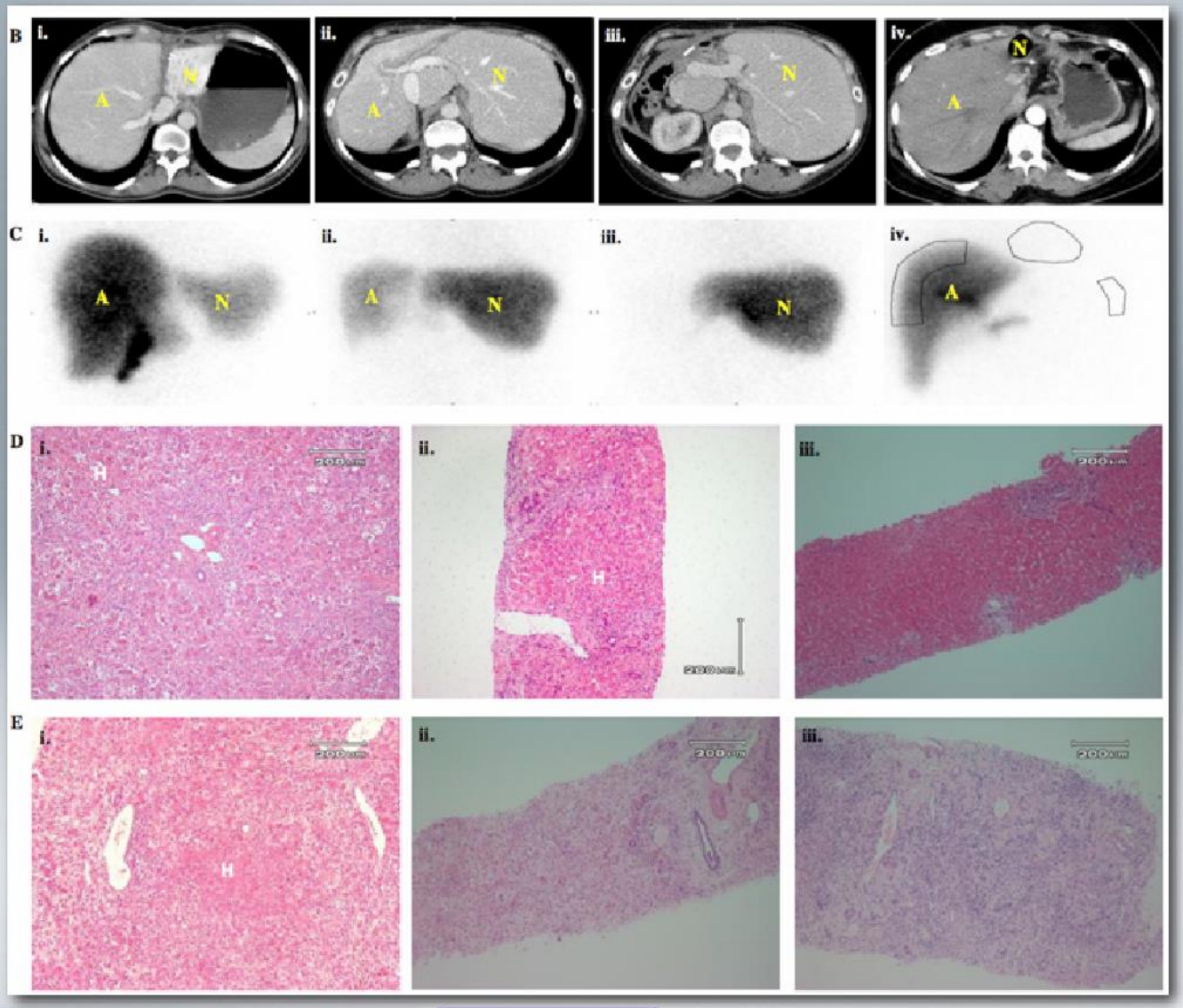


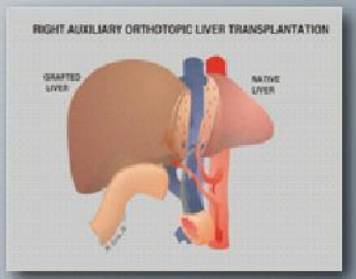


KING'S HEALTH PARTNERS

MRC Centre for Transplantation

AUXILIARY TRANSPLANTATION

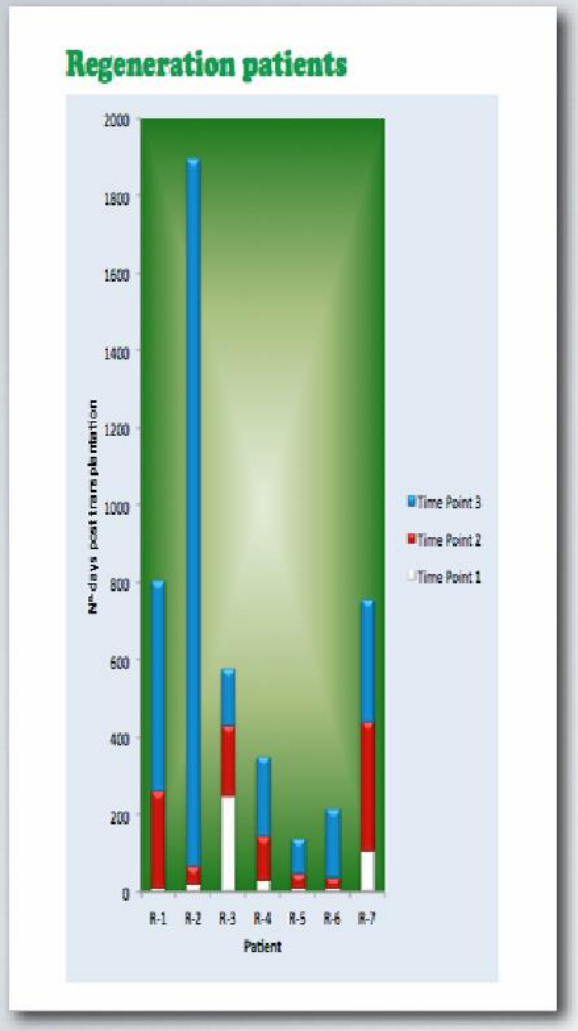




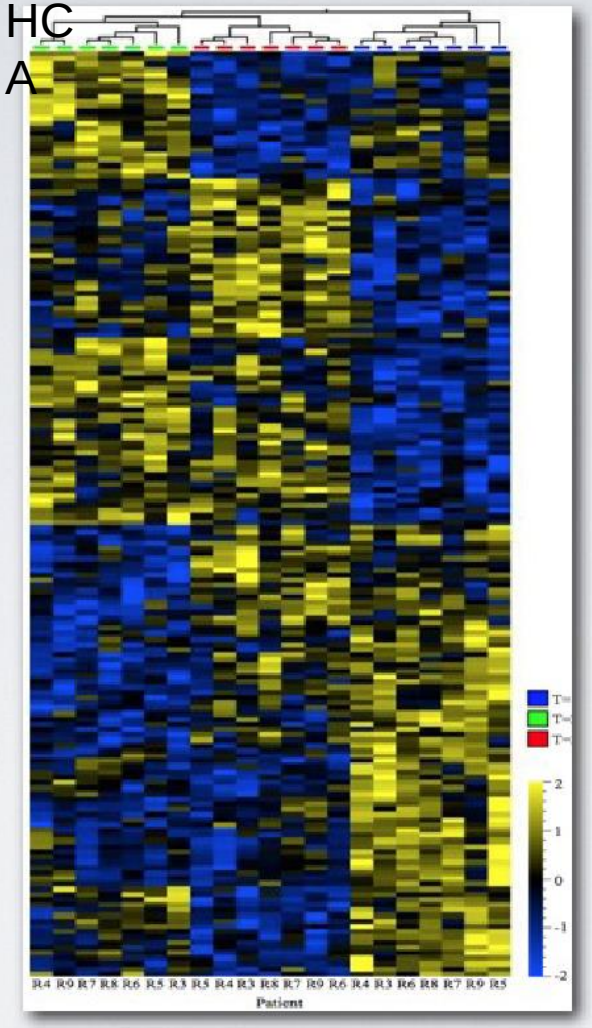
KING'S HEALTH PARTNERS

MRC Centre for Transplantation

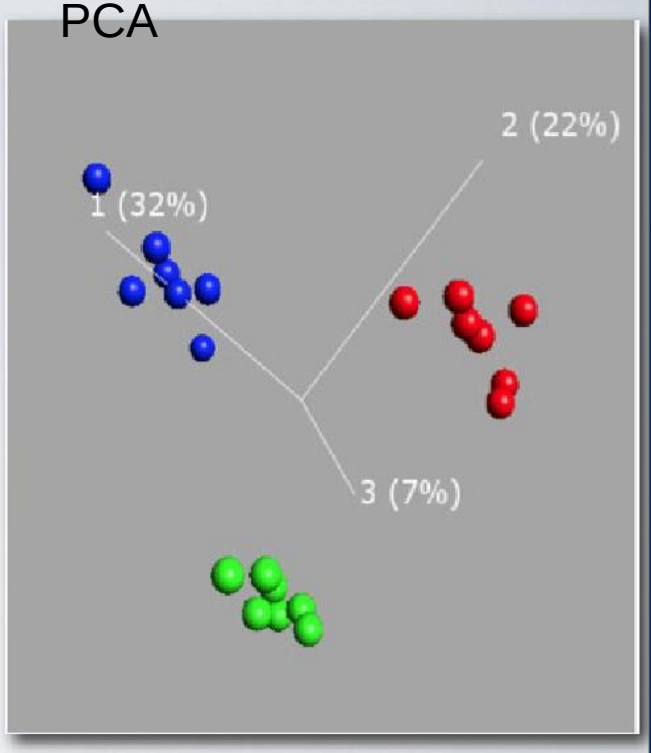
AUXILIARY TRANSPLANT *NATIVE* LIVER '*REGENERATORS*' T0, T1 AND T2



HC
A



PCA

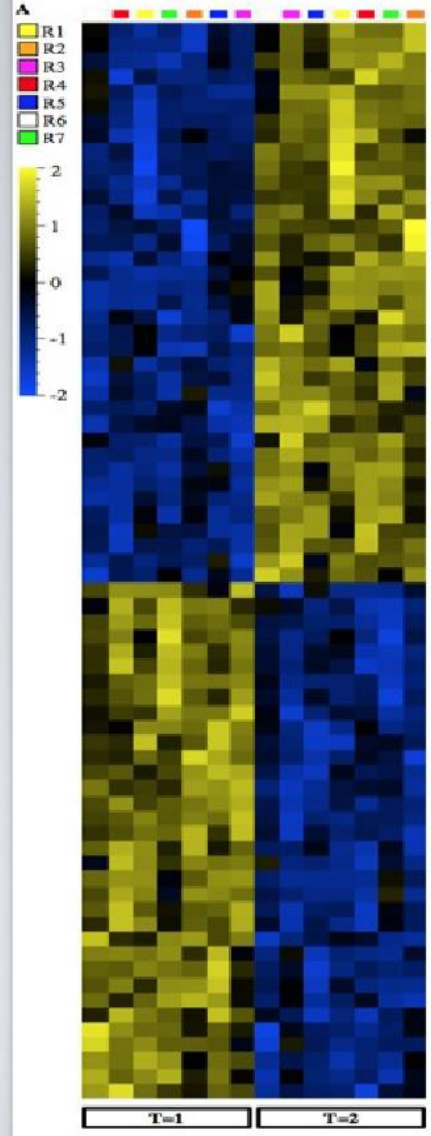


KING'S HEALTH PARTNERS

MRC Centre for Transplantation

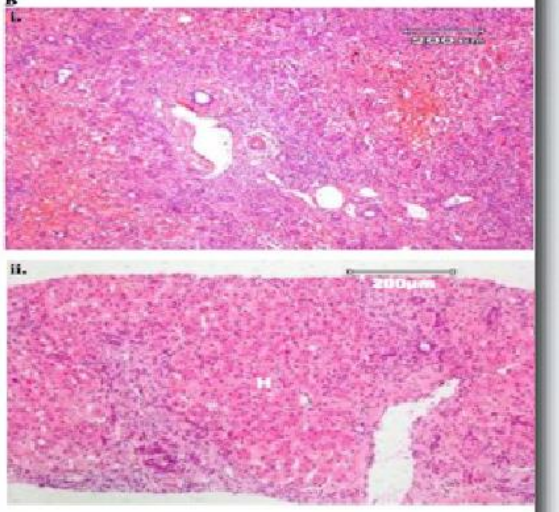
AUXILIARY TRANSPLANT *NATIVE* LIVER '*REGENERATORS*' T0 VS T1

A



miRNA	*P* value
tae-MIR167	0.0067
hsa-mir-624	0.0059
HBII-82	0.0023
mgU6-77	0.0075
cel-mir-790	0.0054
cre-MIR1166	0.0041
osa-MIR821b	0.0006
ptr-mir-136	0.0099
ENSG00000206976	0.0016
hsa-mir-512-1/-2	0.0047
mml-mir-33a	0.0016
mmu-mir-330	0.0042
mmu-mir-409	0.0020
ENSG00000212618	0.0081
ENSG00000200999	0.0078
ENSG00000201025	0.0068
osa-MIR821b	0.0006
zma-MIR169i	0.0093
ENSG00000212529	0.0021
age-mir-34a	0.0089
rno-mir-20a	0.0045
hsa-mir-520e	0.0041
sme-mir-1b	0.0084
ptc-MIR159d	0.0018
xtr-mir-29b	0.0094
ppt-mir1064	0.0051
HBII-85-26	0.0060
sme-mir1082b	0.0035
ENSG00000202498	0.0004
xtr-mir-16b	0.0046
cfa-mir-429	0.0040
rno-mir-130a	0.0010
ENSG00000202389	0.0062
sla-mir-198	0.0094
ath-MIR832	0.0006
ENSG00000202216	0.0094
mmu-mir-126	0.0088
ptc-MIR171e	0.0086
mmu-mir-467e	0.0098
cfa-mir-500	0.0006
gga-mir-29b-1 /-2	0.0079
mmu-mir-92a-1 /-2	0.0054
snR38B	0.0041
dme-mir-315	0.0009
ath-MIR166c	0.0051
hsa-mir-23a	0.0060
ppa-mir-29a	0.0071
mml-mir-22	0.0078
mmu-mir-503	0.0061
hsa-mir-1308	0.0030
mmu-mir-23a	0.0026
xtr-mir-367	0.0002
zma-MIR167d	0.0003
osa-MIR169h	0.0023
dps-mir-92a	0.0044
ath-MIR166f	0.0087
mml-mir-136	0.0026
hsa-mir-654	0.0073
osa-MIR167f	0.0028
sme-mir166b	0.0066
xtr-mir-9a-1 /-2	0.0037
hsa-mir-150	0.0056
ACA59	0.0011
hsa-mir-643	0.0048
aga-mir-1174	0.0047
rno-mir-24-1 /-2	0.0063
xtr-mir-200a	0.0086
hsa-mir-371	0.0090
tae-mir1128	0.0023
cbr-mir-74	0.0055
hsa-mir-663	0.0056

B



C, i.

Network	*P* value	*Z*	*G*
miR-503, miR-23a, miR-150, miR-663, miR-654	4.95e-19	75	75

ii.

miRNA	Target gene
↓miR-503	↑ Cyclin D1● / Cyclin E2● / Cyclin F●/ C Wee1● / CDC25A● / CHK1● / CDKN1B●
↓miR-23a	↑ TNFα● / c-Myc●
↓miR-150	↑ TNFα● / Survivin● / FoxP1● / c-Myb●
↓miR-663	↑ TGF-β1● / JunB● / JunD● / AP-1●

D, i

Network	*P* value	*Z*	*G*
miR-126, miR-130a, miR-20a, miR-520e, miR-330	5.57e-19	80	80

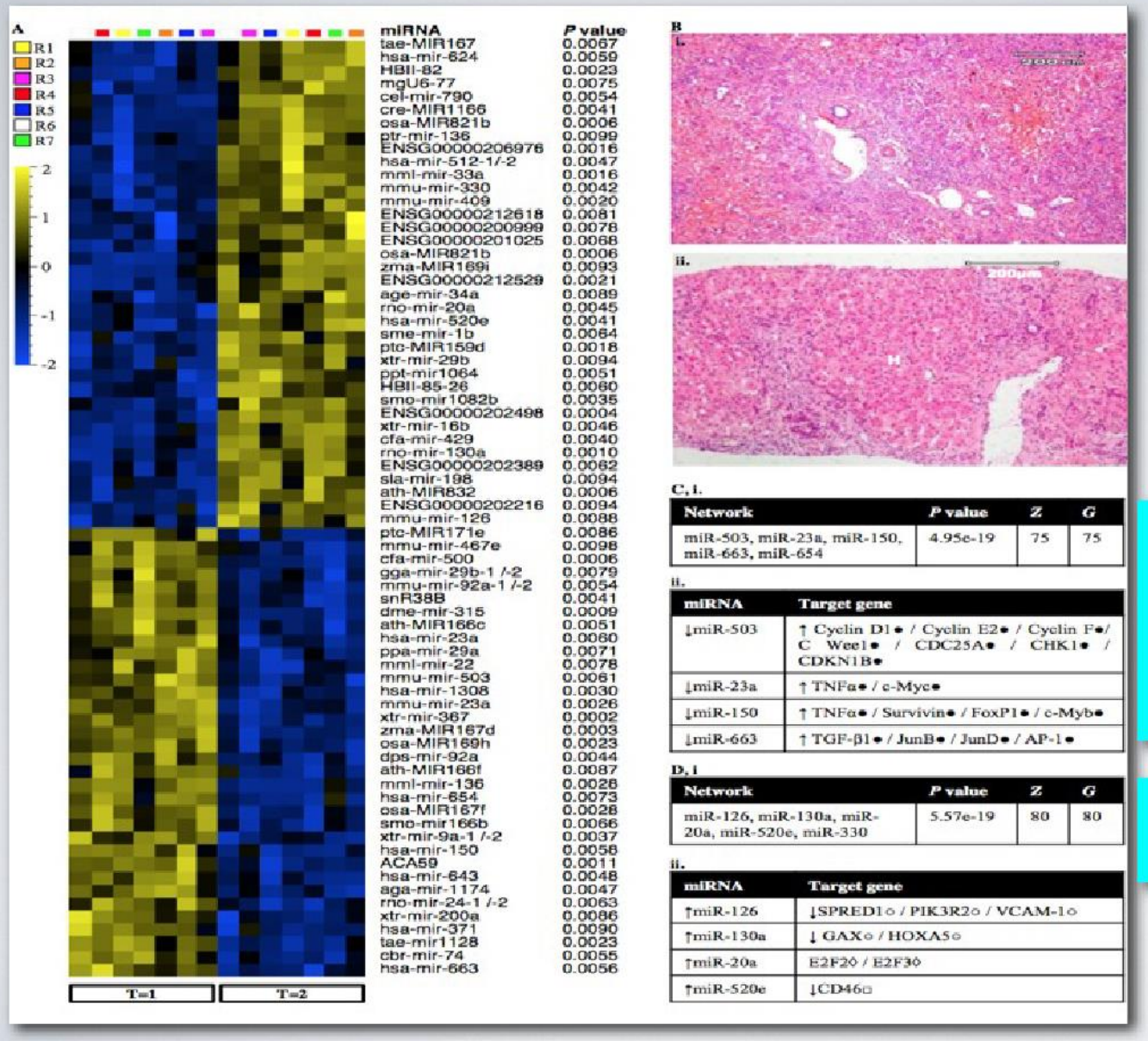
ii.

miRNA	Target gene
↑miR-126	↓SPRED1○ / PIK3R2○ / VCAM-1○
↑miR-130a	↓ GAX○ / HOXA5○
↑miR-20a	E2F2◊ / E2F3◊
↑miR-520e	↓CD46□

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

AUXILIARY TRANSPLANT *NATIVE* LIVER '*REGENERATORS*' T0 VS T1



pathways activated by coordinated downregulation of these miRNA include those known to be critical for the early phase of liver regeneration
by promoting hepatocyte proliferation.

Cell cycle

Inflammation

Anti-apoptotic

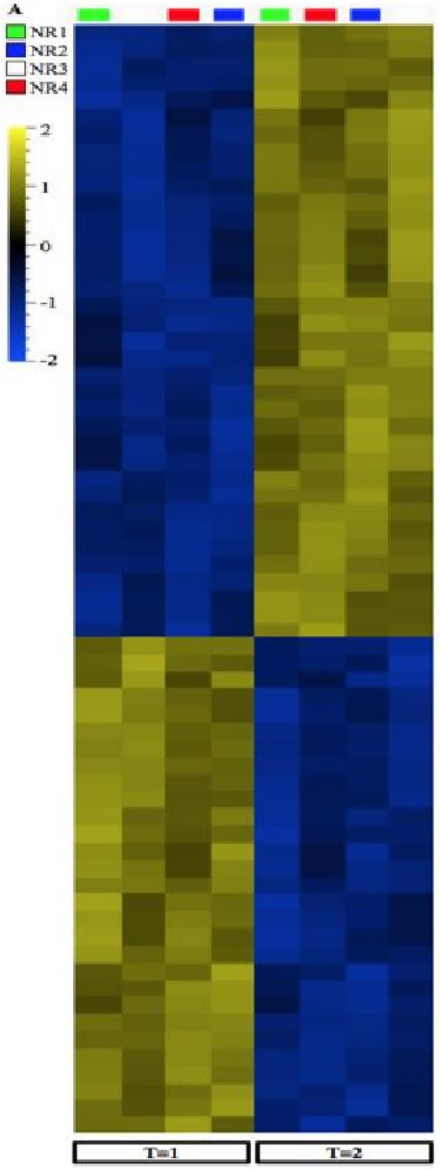
Angiogenesis
Innate Immunity

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

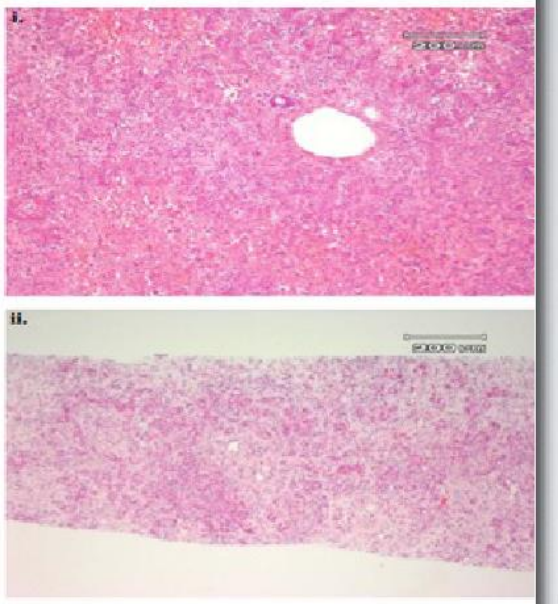
AUXILIARY TRANSPLANT *NATIVE* LIVER '*NON-REGENERATORS*' T0 VS T1

A



miRNA	*P* value
mghv-mir-M1-1	0.000517
mo-mir-17-1/-2	0.001704
hsa-let-7i	0.002299
eel-mir-70	0.001376
rlev-mir-rl1-12	0.009968
odi-mir-1497f	0.00828
ggo-mir-135	0.004016
ptc-MIR171b	0.003113
sbi-mir169i	0.001798
vvi-MIR395j	0.00373
ENSG00000199321	0.001925
vvi-MIR390	0.00173
osa-MIR806g	0.007783
xtr-mir-193	0.006521
ENSG00000199474	0.01
bta-mir-192	0.003262
xtr-mir-93b	0.001259
eel-mir-241	0.007396
mml-mir-140	0.007182
tni-mir-21	0.008054
ENSG00000201448	9.40E-05
zma-MIR160e	0.002433
tae-MIR159a	0.001677
mml-mir-649	0.004335
hsa-mir-558	0.008534
dme-mir-276	0.00399
hsa-mir-1278	0.001434
bta-mir-30e	0.003183
ENSG00000207047	0.001738
odi-mir-1483	0.003485
mo-mir-150	0.0021
U50B	0.000971
mml-mir-451	0.002315
cre-MIR1152	0.0061
mdo-mir-223	0.005843
aga-mir-34	0.006
tni-mir-218a-1/-2	0.002051
ppy-mir-143	0.00655
hsa-mir-516b-2/-1	0.009428
mml-mir-671	0.005158
ppy-mir-32	0.004942
xtr-mir-206	0.001514
gga-mir-367	0.001644
mtr-mir169b	0.001285
mmu-mir-883b	0.003212
ath-MIR854d	0.005463
age-mir-508	0.00313
U109	0.006325
hsa-mir-7-2/-3/-1	0.009161
sbi-mir395f	0.005682
mdo-mir-375	0.000613
cin-mir-183	0.008551
xtr-mir-301-2/-1	0.005048
hsa-mir-152	0.009375
dme-mir-977	0.003083
odi-mir-1487	0.005137
ENSG00000206898	0.006012
mdv2-mir-m20	0.006286
odi-mir-1b	0.000752
ebr-mir-42	0.000994
ggo-mir-199a	0.001252
ppa-mir-147	0.001874
ptc-MIR169y	0.003227
ENSG00000199600	0.004005
gma-mir156e	0.003443

B



C, i

Network	*P* value	*Z*	*G*
miR-152, DNMT1, AML1/ETO fusion protein, c-FLIP (L), ITGAL	1.59e-04	80	80

ii

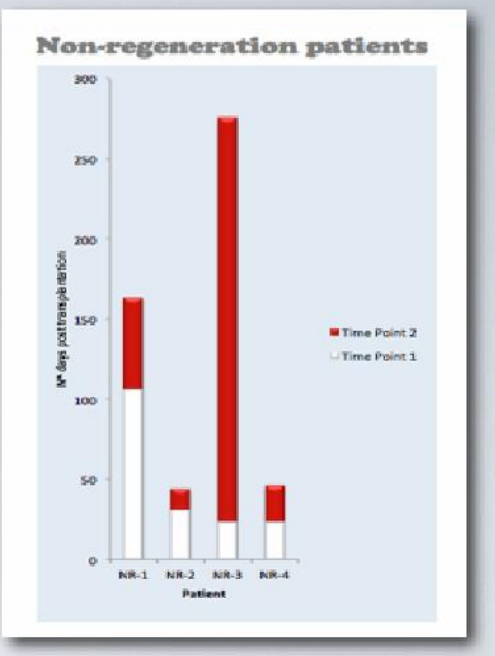
miRNA	Target gene
↓miR-152	↑DNMT1●

D, i

Network	*P* value	*Z*	*G*
miR-150, let-7i, HBII-52 snoRNA, c-Src, C/EBPbeta	6.48e-09	47	47

ii

miRNA	Target gene
↑miR-150	↓TNFα● / Survivin● / FoxP1● / c-Myb●
↑ let-7i	↓ TLR4□

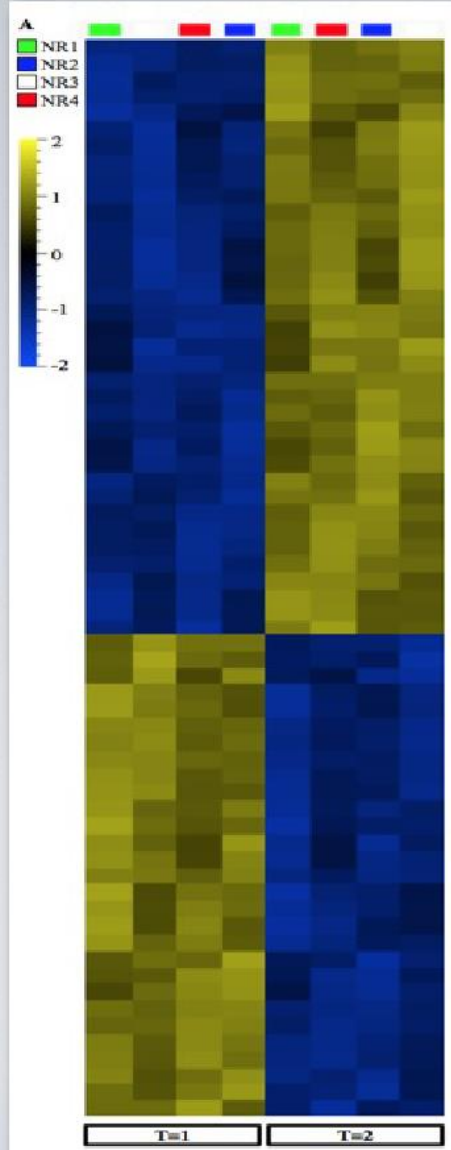


KING'S HEALTH PARTNERS

MRC Centre for Transplantation

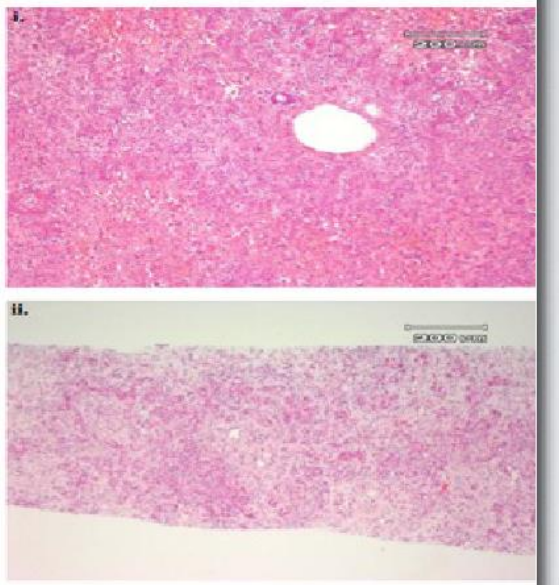
AUXILIARY TRANSPLANT *NATIVE* LIVER '*NON-REGENERATORS*' T0 VS T1

A



miRNA	*P* value
mghv-mir-M1-1	0.000517
mo-mir-17-1/-2	0.001704
hsa-let-7i	0.002299
cel-mir-70	0.001376
rlev-mir-rl1-12	0.009968
odi-mir-1497f	0.00828
ggo-mir-135	0.004016
ptc-MIR171b	0.003113
sbi-mir169i	0.001798
vvi-MIR395j	0.00373
ENSG00000199321	0.001925
vvi-MIR390	0.00173
osa-MIR806g	0.007783
xtr-mir-193	0.006521
ENSG00000199474	0.01
bta-mir-192	0.003262
xtr-mir-93b	0.001259
cel-mir-241	0.007396
mml-mir-140	0.007182
tni-mir-21	0.008054
ENSG00000201448	9.40E-05
zma-MIR160e	0.002433
tae-MIR159a	0.001677
mml-mir-649	0.004335
hsa-mir-558	0.008534
dme-mir-276	0.00399
hsa-mir-1278	0.001434
bta-mir-30e	0.003183
ENSG00000207047	0.001738
odi-mir-1483	0.003485
mo-mir-150	0.0021
U50B	0.000971
mml-mir-451	0.002315
cre-MIR1152	0.0061
mdo-mir-223	0.005843
aga-mir-34	0.006
tni-mir-218a-1/-2	0.002051
ppy-mir-143	0.00655
hsa-mir-516b-2/-1	0.009428
mml-mir-671	0.005158
ppy-mir-32	0.004942
xtr-mir-206	0.001514
gga-mir-367	0.001644
mtr-mir169b	0.001285
mmu-mir-883b	0.003212
ath-MIR854d	0.005463
age-mir-508	0.00313
U109	0.006325
hsa-mir-7-2/-3/-1	0.009161
sbi-mir395f	0.005682
mdo-mir-375	0.000613
cin-mir-183	0.008551
xtr-mir-301-2/-1	0.005048
hsa-mir-152	0.009375
dme-mir-977	0.003083
odi-mir-1487	0.005137
ENSG00000206898	0.006012
mdv2-mir-m20	0.006286
odi-mir-1b	0.000752
ebr-mir-42	0.000994
ggo-mir-199a	0.001252
ppa-mir-147	0.001874
ptc-MIR169y	0.003227
ENSG00000199600	0.004005
gma-mir156e	0.003443

B



C, i

Network	*P* value	*Z*	*G*
miR-152, DNMT1, AML1/ETO fusion protein, c-FLIP (L), ITGAL	1.59e-04	80	80

ii

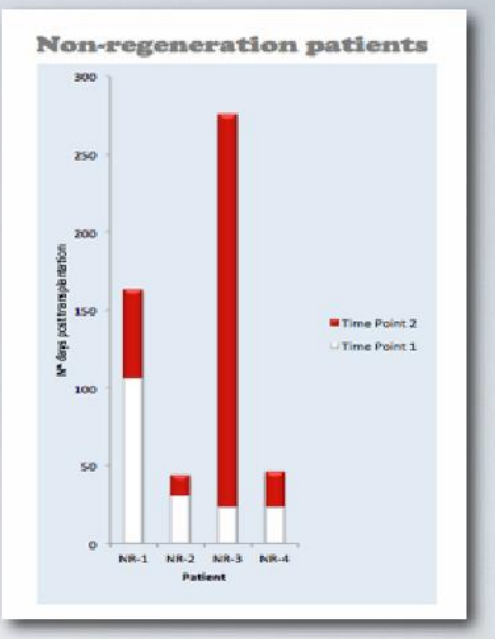
miRNA	Target gene
↓miR-152	↑DNMT1○

D, i

Network	*P* value	*Z*	*G*
miR-150, let-7i, HBII-52 snoRNA, c-Src, C/EBPbeta	6.48e-09	47	47

ii

miRNA	Target gene
↑miR-150	↓TNFα● / Survivin● / FoxP1● / c-Myb●
↑ let-7i	↓ TLR4□



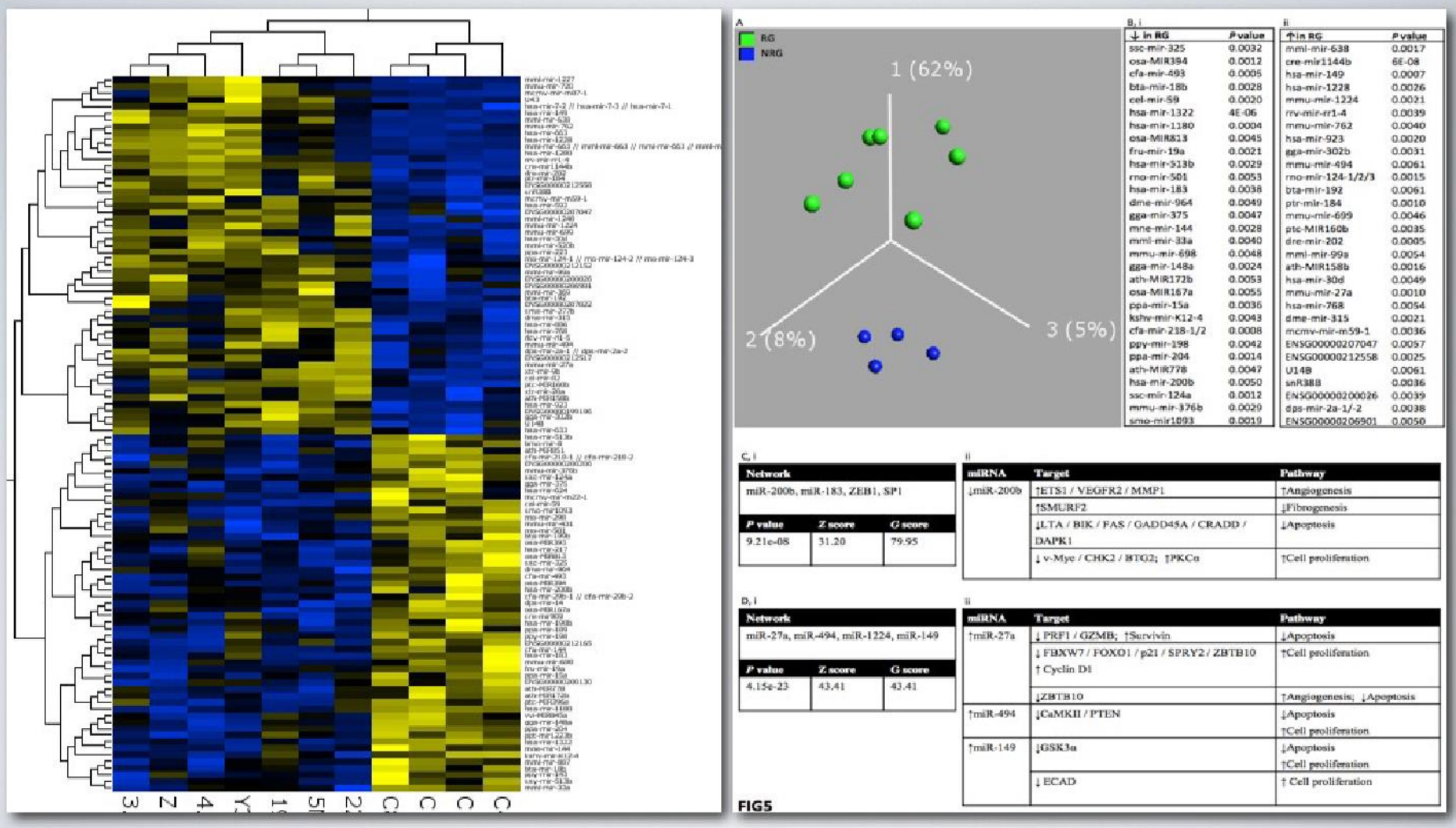
Global DNA methylation

Cell cycle inhibition
Reduced inflammation
Pro-apoptotic

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

AUXILIARY TRANSPLANT *NATIVE* LIVER T0
'REGENERATORS' VS *'NON-REGENERATORS'*



B, i

↓ in RG	P value
ssc-mir-325	0.0032
osa-MIR394	0.0012
cfa-mir-493	0.0005
bta-mir-18b	0.0028
cel-mir-59	0.0020
hsa-mir-1322	4E-06
hsa-mir-1180	0.0004
osa-MIR813	0.0045
fru-mir-19a	0.0021
hsa-mir-513b	0.0029
rno-mir-501	0.0053
hsa-mir-183	0.0038
dme-mir-964	0.0049
gga-mir-375	0.0047
mne-mir-144	0.0028
mml-mir-33a	0.0040
mmu-mir-698	0.0048
gga-mir-148a	0.0024
ath-MIR172b	0.0053
osa-MIR167a	0.0055
ppa-mir-15a	0.0036
kshv-mir-K12-4	0.0043
cfa-mir-218-1/2	0.0008
ppy-mir-198	0.0042
ppa-mir-204	0.0014
ath-MIR778	0.0047
hsa-mir-200b	0.0050
ssc-mir-124a	0.0012
mmu-mir-376b	0.0029
sme-mir1893	0.0019

ii

↑ in RG	P value
mml-mir-638	0.0017
cre-mir1144b	6E-08
hsa-mir-149	0.0007
hsa-mir-1228	0.0026
mmu-mir-1224	0.0021
rrv-mir-rr1-4	0.0039
mmu-mir-762	0.0040
hsa-mir-923	0.0020
gga-mir-302b	0.0031
mmu-mir-494	0.0061
rno-mir-124-1/2/3	0.0015
bta-mir-192	0.0061
ptr-mir-184	0.0010
mmu-mir-699	0.0046
ptc-MIR160b	0.0035
dre-mir-202	0.0005
mml-mir-99a	0.0054
ath-MIR158b	0.0016
hsa-mir-30d	0.0049
mmu-mir-27a	0.0010
hsa-mir-768	0.0054
dme-mir-315	0.0021
mcmv-mir-m59-1	0.0036
ENSG00000207047	0.0057
ENSG00000212558	0.0025
U148	0.0061
snR38B	0.0036
ENSG00000200026	0.0039
dps-mir-2a-1/-2	0.0038
ENSG00000206901	0.0050

C, i

Network		
miR-200b, miR-183, ZEB1, SP1		
P value	**Z score**	**G score**
9.21e-08	31.20	79.95

ii

miRNA	Target	Pathway
↓miR-200b	↑ETS1 / VEGFR2 / MMP1	↑Angiogenesis
	↑SMURF2	↓Fibrogenesis
	↓LTA / BIK / FAS / GADD45A / CRADD / DAPK1	↓Apoptosis
	↓ v-Myc / CHK2 / BTG2; ↑PKCα	↑Cell proliferation

D, i

Network		
miR-27a, miR-494, miR-1224, miR-149		
P value	**Z score**	**G score**
4.15e-23	43.41	43.41

ii

miRNA	Target	Pathway
↑miR-27a	↓ PRF1 / GZMB; ↑Survivin	↓Apoptosis
	↓ FBXW7 / FOXO1 / p21 / SPRY2 / ZBTB10 ↑ Cyclin D1	↑Cell proliferation
	↓ZBTB10	↑Angiogenesis; ↓Apoptosis
↑miR-494	↓CaMKII / PTEN	↓Apoptosis ↑Cell proliferation
↑miR-149	↓GSK3α	↓Apoptosis ↑Cell proliferation
	↓ ECAD	↑ Cell proliferation

FIG5

The distinct miRNA expression patterns between the RG and NRG at the time of ALT indicate that regeneration had already commenced at T1 in the RG.

IN VITRO MANIPULATION I

- *Lentiviral transduction*
- *Primary hepatocytes*

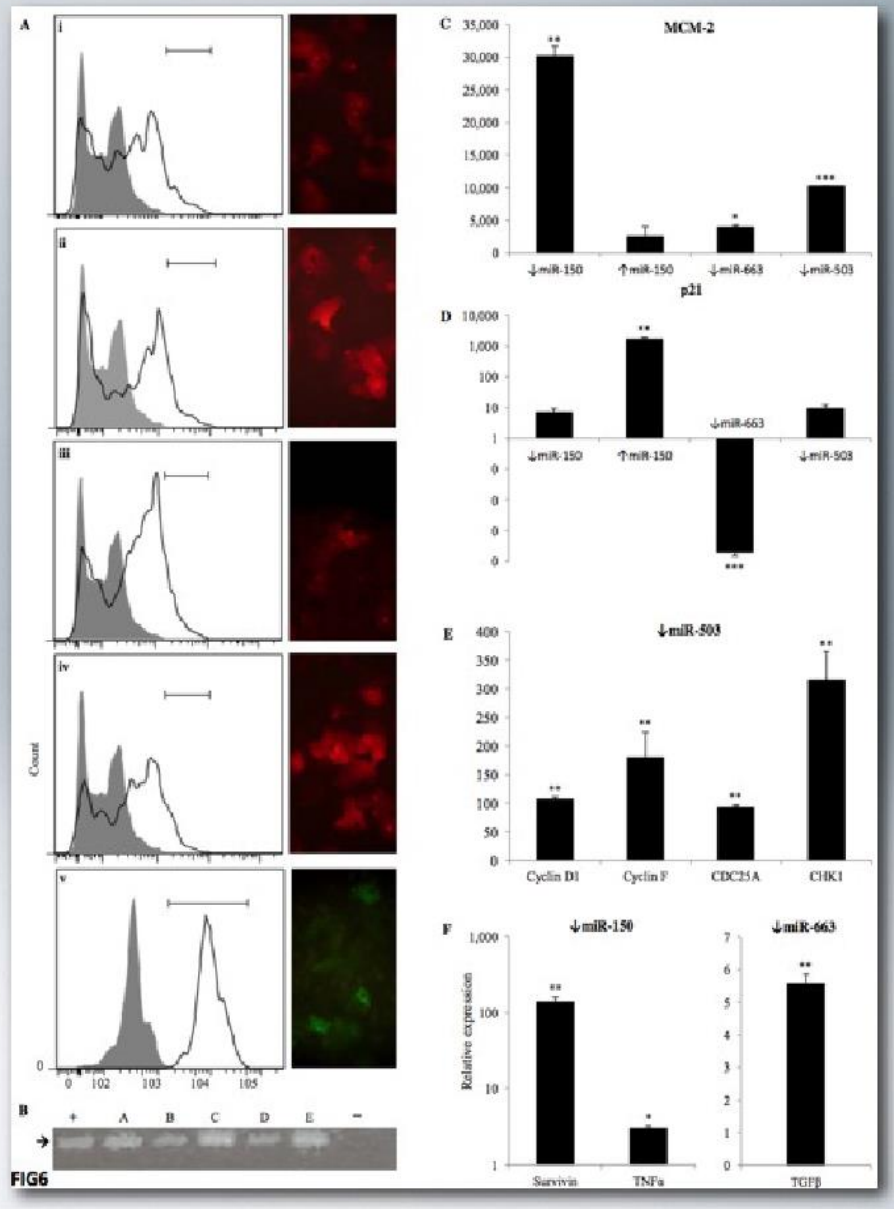


FIG6

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

IN VITRO MANIPULATION I

- *Cell cycle competent*
- *Upregulation of known target genes*
- *Cell cycle genes associated with regeneration*

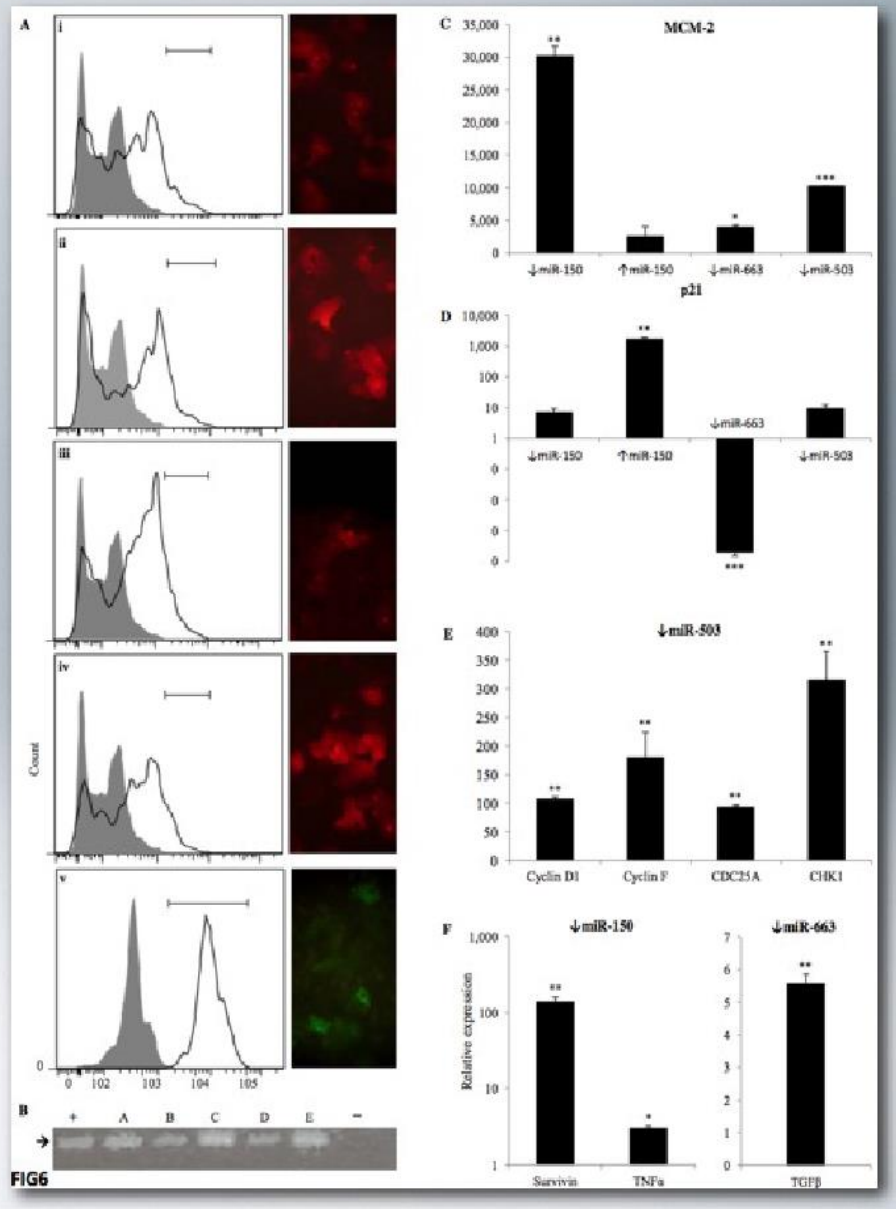


FIG6

MCM2: minichromosome

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

IN VITRO MANIPULATION 2

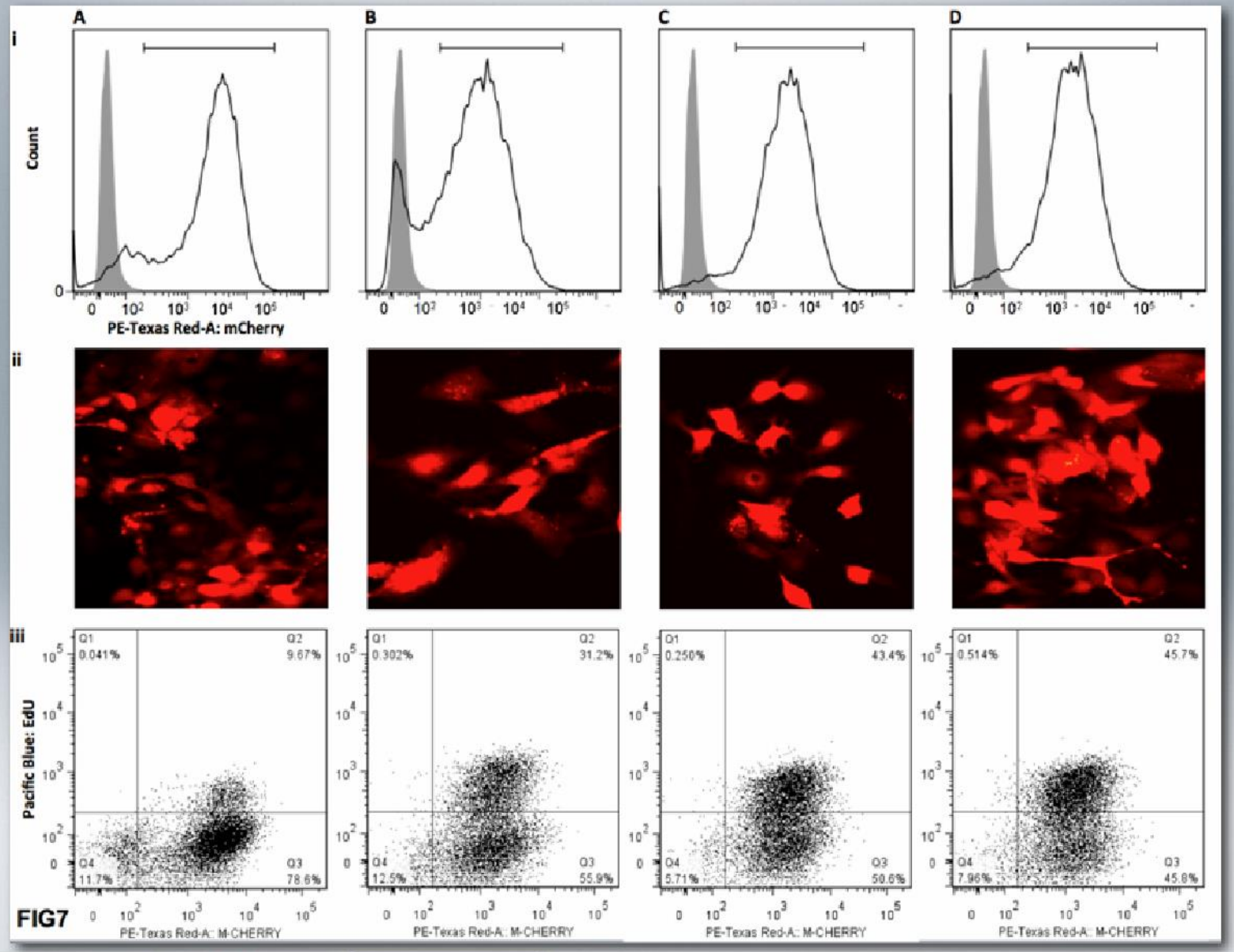


FIG7

KING'S HEALTH PARTNERS

MRC Centre for Transplantation

The team have received funding to look for those chemical differences in the blood of patients.

Human Liver Regeneration Is Characterized by the Coordinated Expression of Distinct MicroRNA Governing Cell Cycle Fate

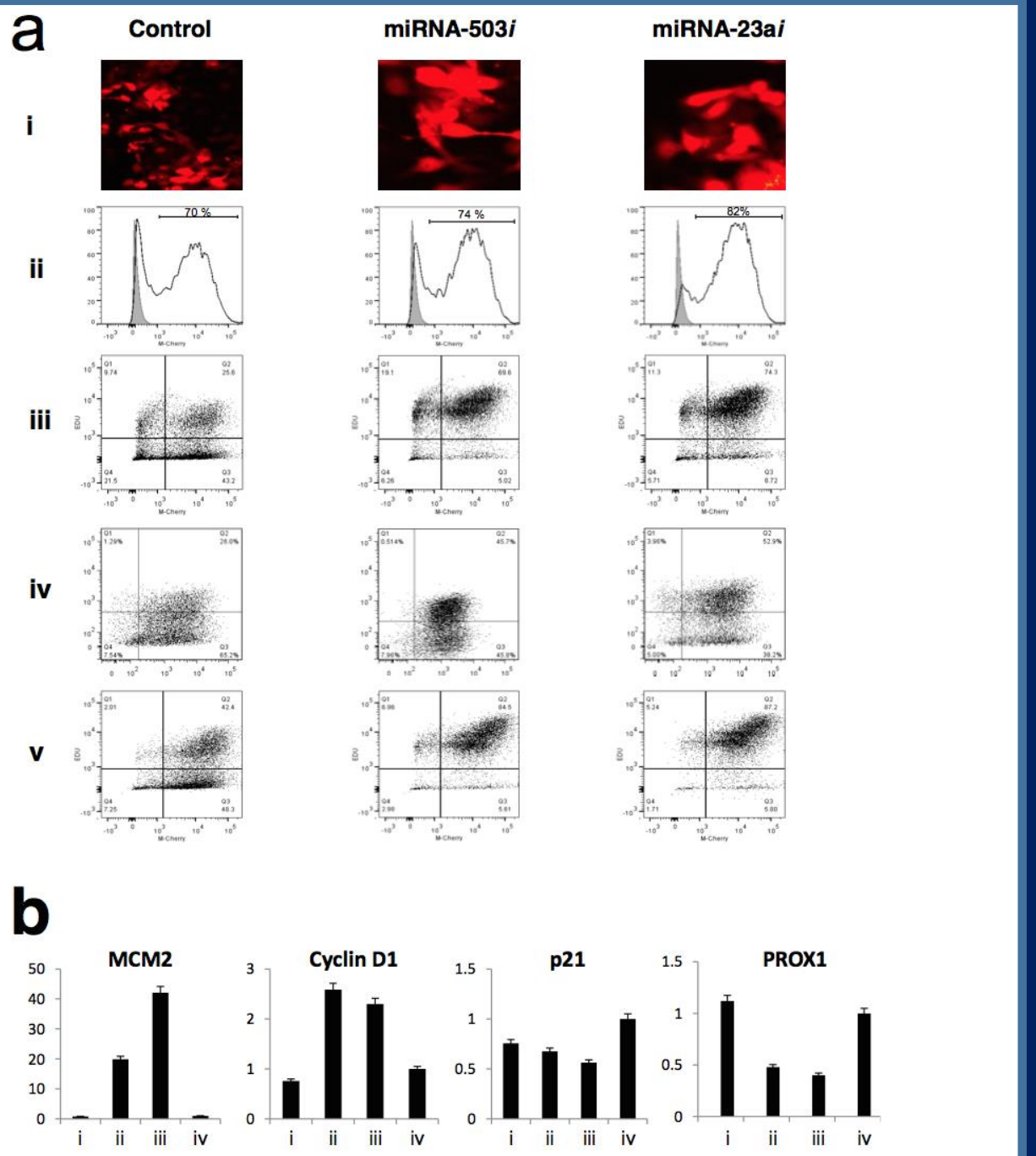
Salehi, S., Brereton, H. C., Arno, M. J., Darling, D., Quaglia, A., O'Grady, J., Heaton, N. & Aluvihare, V. R., May 2013, In : American Journal of Transplantation. 13, 5, p. 1282-1295Article

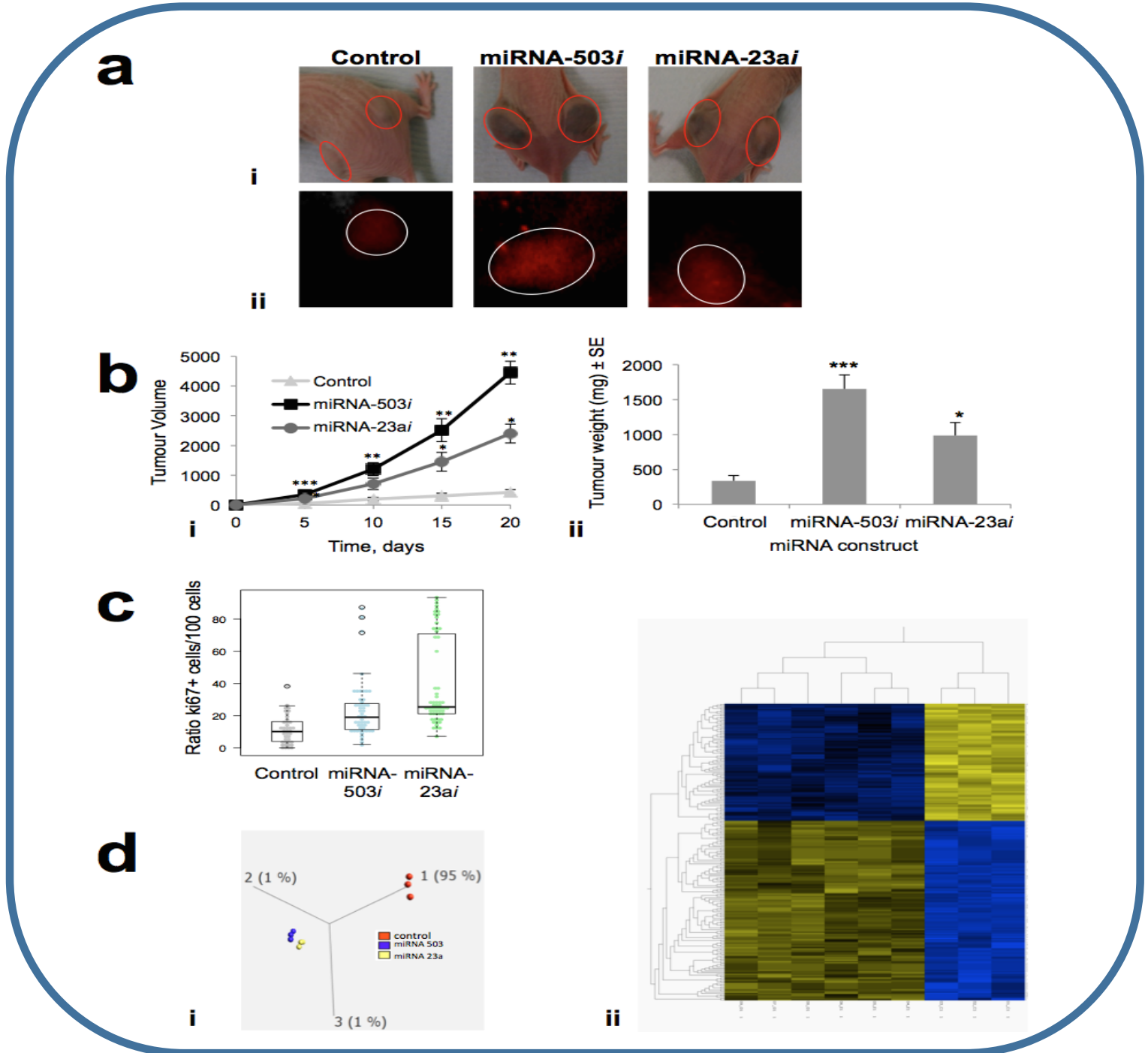
microRNA regulating regenerative capacity alter tumour aggression and can enforce tumour growth arrest in vivo

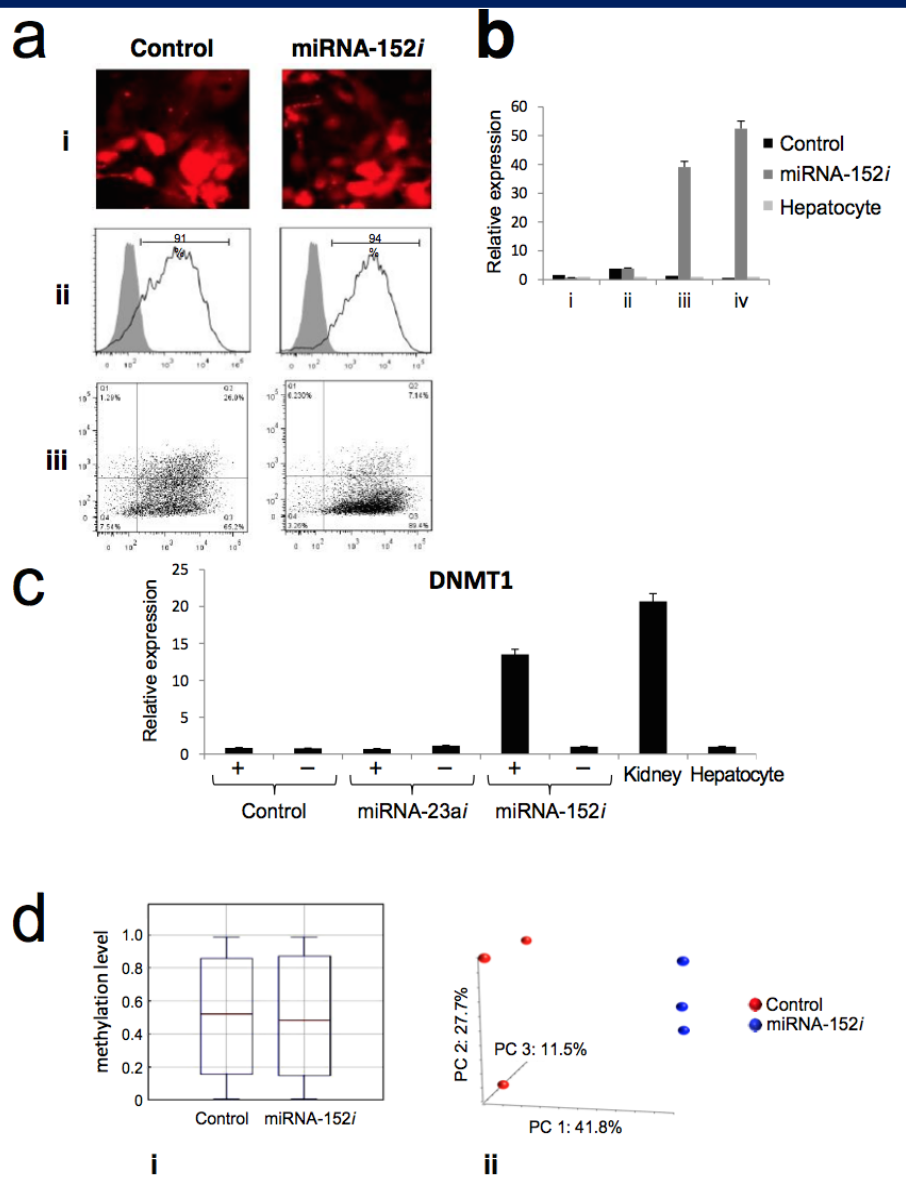
Siamak Salehi[1], Helen C Brereton[1], Augusto Villanueva[1], Kosh Agrawal[1], Julie Watson[2], David Darling[3], Farzin Farzaneh[3], Alberto Quaglia[1], Nigel Heaton[1,4] and Varuna R Aluvihare[1,4]

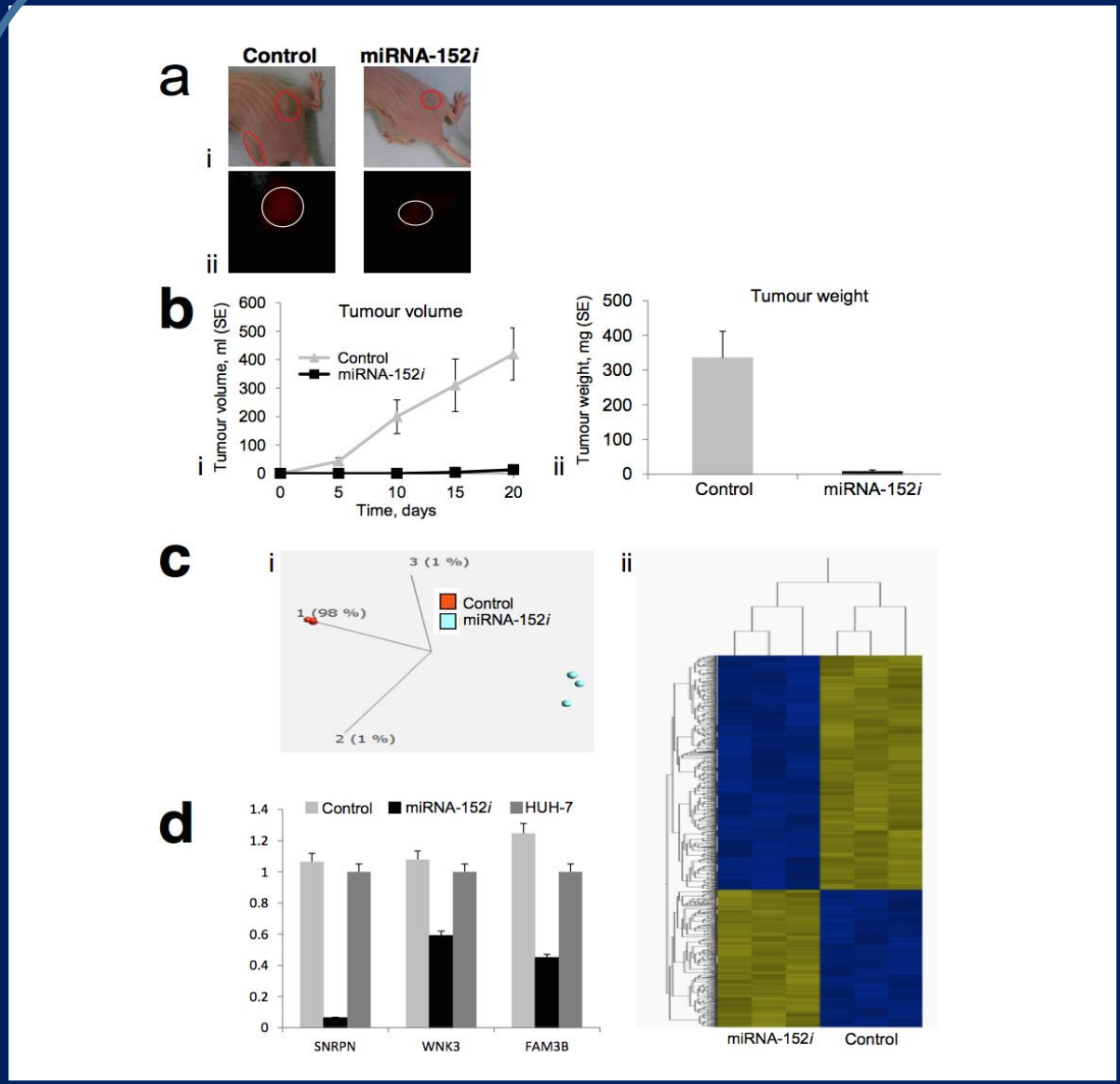
[1]Institute of Liver Studies, King's College Hospital, London, United Kingdom
[2]MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, King's College London, Guy's Hospital London, United Kingdom
[3]Department of Hematological and Molecular Medicine, King's College London, London, United Kingdom
[4]MRC Transplant Centre, King's College London, London, United Kingdom

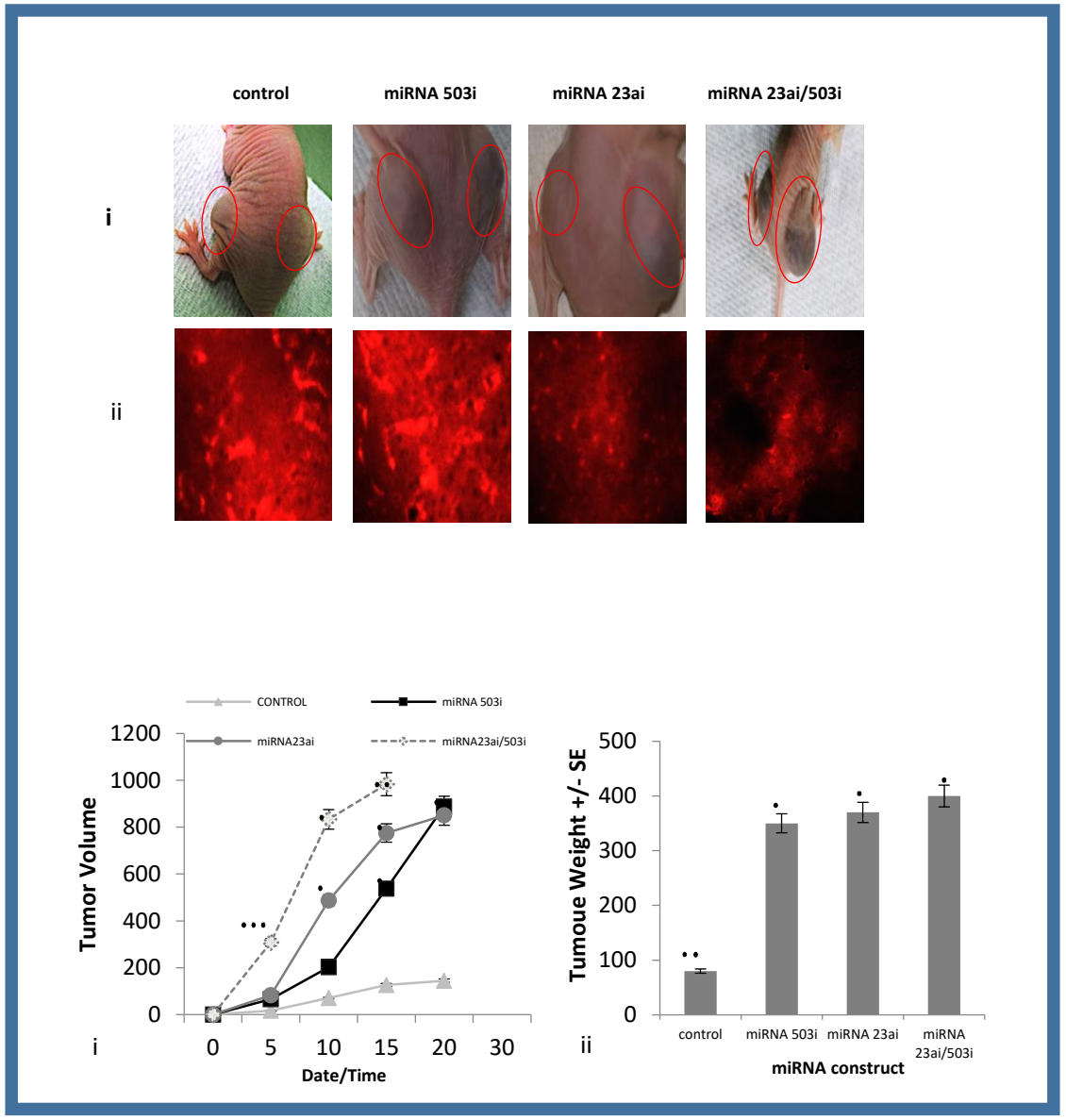
Nature Medicine Sub Sep 2019

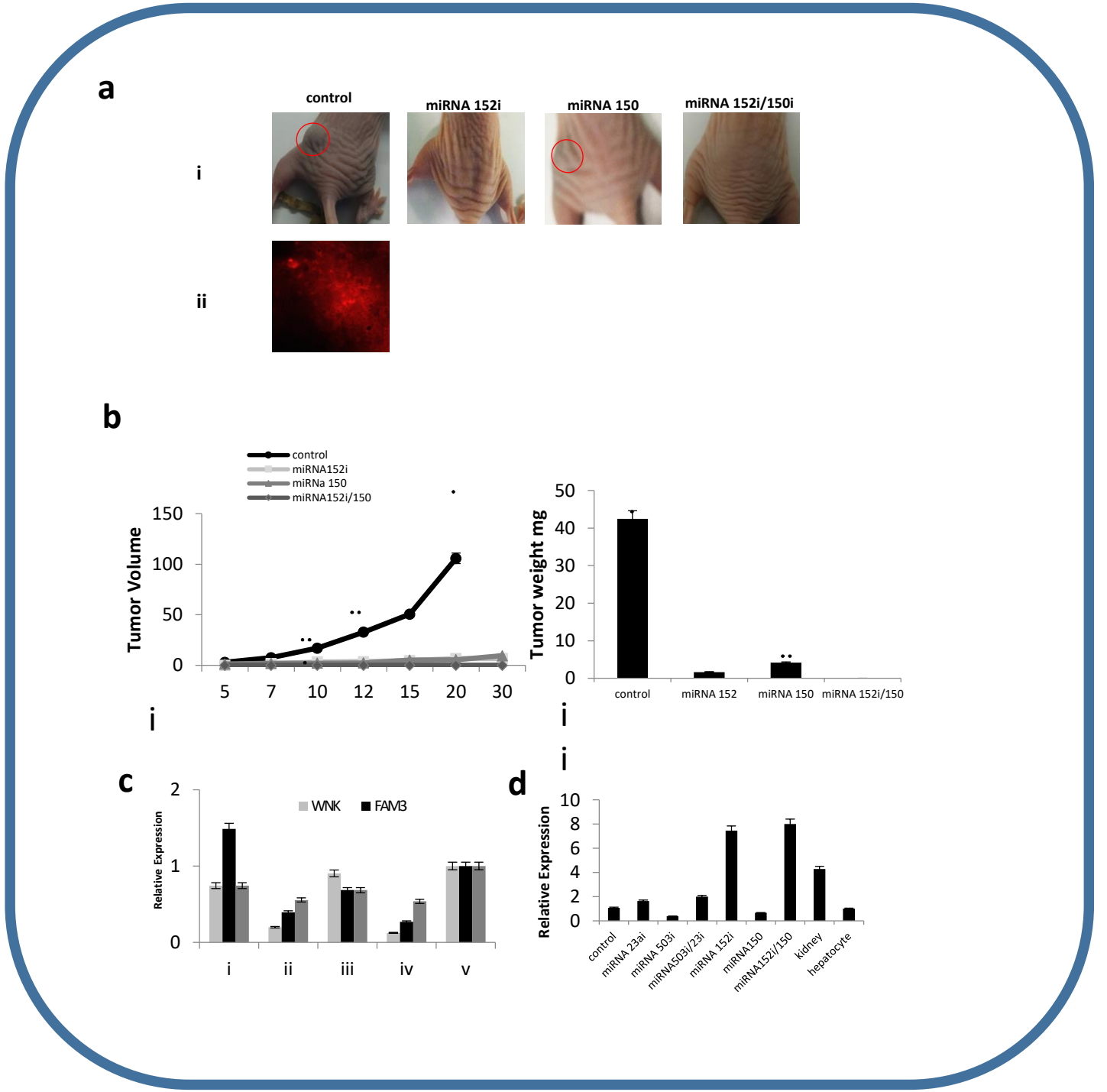

a
Control
miRNA-503i
miRNA-23ai
i
ii
iii
iv
v
70 %
74 %
82%
b
MCM2
Cyclin D1
p21
PROX1
i
ii
iii
iv


a
Control
miRNA-503i
miRNA-23ai
i
ii
b
Tumour Volume
5000
4000
3000
2000
1000
0
Control
miRNA-503i
miRNA-23ai
0
5
10
15
20
Time, days
i
Tumour weight (mg) ± SE
2000
1500
1000
500
0
Control
miRNA-503i
miRNA-23ai
miRNA construct
ii
c
Ratio ki67+ cells/100 cells
80
60
40
20
0
Control
miRNA-503i
miRNA-23ai
d
2 (1 %)
1 (95 %)
3 (1 %)
control
miRNA 503
miRNA 23a
i
ii


a
Control
miRNA-152i
i
ii
iii
91
94
b
Relative expression
60
50
40
30
20
10
0
i
ii
iii
iv
Control
miRNA-152i
Hepatocyte
c
DNMT1
Relative expression
25
20
15
10
5
0
+
−
+
−
+
−
Kidney
Hepatocyte
Control
miRNA-23ai
miRNA-152i
d
methylation level
1.0
0.8
0.6
0.4
0.2
0
Control
miRNA-152i
i
PC 2: 27.7%
PC 3: 11.5%
PC 1: 41.8%
Control
miRNA-152i
ii


a
Control
miRNA-152i
i
ii
b
Tumour volume
Tumour volume, ml (SE)
Control
miRNA-152i
Time, days
Tumour weight
Tumour weight, mg (SE)
Control
miRNA-152i
c
3 (1 %)
1 (98 %)
2 (1 %)
Control
miRNA-152i
d
Control
miRNA-152i
HUH-7
SNRPN
WNK3
FAM3B
miRNA-152i
Control


control
miRNA 503i
miRNA 23ai
miRNA 23ai/503i
i
ii
CONTROL
miRNA 503i
miRNA23ai
miRNA23ai/503i
Tumor Volume
1200
1000
800
600
400
200
0
0
5
10
15
20
30
Date/Time
i
Tumoue Weight +/- SE
500
400
300
200
100
0
control
miRNA 503i
miRNA 23ai
miRNA 23ai/503i
miRNA construct
ii


a
control
miRNA 152i
miRNA 150
miRNA 152i/150i
i
ii
b
control
miRNA152i
miRNa 150
miRNA152i/150
Tumor Volume
150
100
50
0
5
7
10
12
15
20
30
Tumor weight mg
50
40
30
20
10
0
control
miRNA 152
miRNA 150
miRNA 152i/150
i
i
c
WNK
FAM3
Relative Expression
2
1
0
i
ii
iii
iv
v
d
i
Relative Expression
10
8
6
4
2
0
control
miRNA 23ai
miRNA 503i
miRNA503i/23i
miRNA 152i
miRNA150
miRNA152i/150
kidney
hepatocyte

EDU uptake in HepG2 Transfected cells						
C	G	E	G+E	H	B	H+B
15.3%	17.3%	20.8%	48.1%	13.4%	27.6%	10.6%

EDU uptake in Min6 Transfected cells						
C	G	E	G+E	H	B	H+B
11.2%	12.2%	17.2%	53%	10.4%	16.2%	7.7%

EDU uptake in HUH7 Transfected cells						
C	G	E	G+E	H	B	H+B
58.2%	74.8%	73.3%	78.7%	0%	3.5%	0%

C: Scrambled
G: miRNA 23a inhibitor
E: miRNA 503 inhibitor
H: miRNA 152 inhibitor
B: miRNA 150 expression clone

Our results indicate that regulation of regeneration and tumour aggressiveness are concordant and suggest a potential novel treatment strategy for human cancers based on regulatory inhibitors of regeneration.

Permission to start clinical trial for Cocktail miRNA treatment of HCC and other solid tumours

NHS

King's College Hospital

NHS Foundation Trust

University of San Francisco

Karin Gaensler
Abood Okal
Yimin Shi

University of Palermo

Caterina Gambino
Sonya Vasto
Giuseppina Candore
Calogero Caruso

University College London

Martin Pule
Karl Peggs

UCL / Institute of Child Health

Waseem Qasim
Bobby Gaspar
Adrian Thrasher

University of California Davies

Peter Barry

National Cancer Centre Singapore

Hui Kam Man

UCL / Royal Free

Emma Morris
Hans Stauss

Zhengzhou Academy of Medical Sciences

Zhimin Wang
Zhongxian Zhang
Yaohe Wang

Royal Marsden

Mike Potter

UCL / Royal Free

Claire Bennett
Ronjon Chakraverty

University of Hannover

Renata Stripecke

Kholood Basahel
Camron Brown
Anil Chandrashekran
Glenda Dickson
Manisha Gurung
Sabine Domning
Lajos Floro
Ravin Khinder
Shahid Miah
Safia Morrison
Felix Munkonge
Ruby Quartey Papafio
Rebecca Prue
Ziyadur Rahman
Emiljano Rakaj
Aishea Satney
Carlo Scala
Philip Whall
Katarzyna Wolanska-Hajduk
Nahid Zareian

Dipika Bhudia
Davide Busato
David Darling
Faith Green
Glenda Dickson
Aniekan Etuk
David George Hullah
Meghan Madigan

Linda Barber
Stephen Devereux
Joop Gäken
Shahram Kordasti
Austin Kulasekaraj
Antonio Pagliuca
Victoria Potter
Eric So

Ghulam Mufti

- ***Molecular Oncology***

  Mahvash Tavassoli

Research Oncology

John Maher

Sophie Pappa

James Spicer

Charlotte Graham
Anieszka Jozwik
Ana Martins Metelo

Reuben Benjamin

KING'S College LONDON

gsk GlaxoSmithKline

CANCER RESEARCH UK

Autolus

ECMC

cellectis

CATAPULT Cell and Gene Therapy

Roche

Innovate UK

BBSRC bioscience for the future

EPSRC Pioneering research and skills

Bloodwise The blood cancer research charity

MEDCITY CAMBRIDGE | LONDON | OXFORD

Department of Health

LIFTBioSciences INNATELY CURING CANCER

Rosetrees Trust

KING'S HEALTH PARTNERS
Integrated Cancer Centre

MRC Centre for Transplantation

King's College Hospital NHS
NHS Foundation Trust

Liver Transplant /Liver cancer group

Siamak Salehi
Helen Brereton
Alberto Quaglia
Nigel Heaton
Kosh Agarwal
Deepak Joshi
Ragai Mitry
Anil Dhawan
Oliver Tawabbi
Varuna Aluvihare