NON-MUTATION INHIBITING THERAPY STRATEGIES

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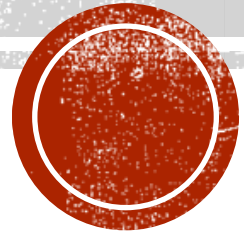
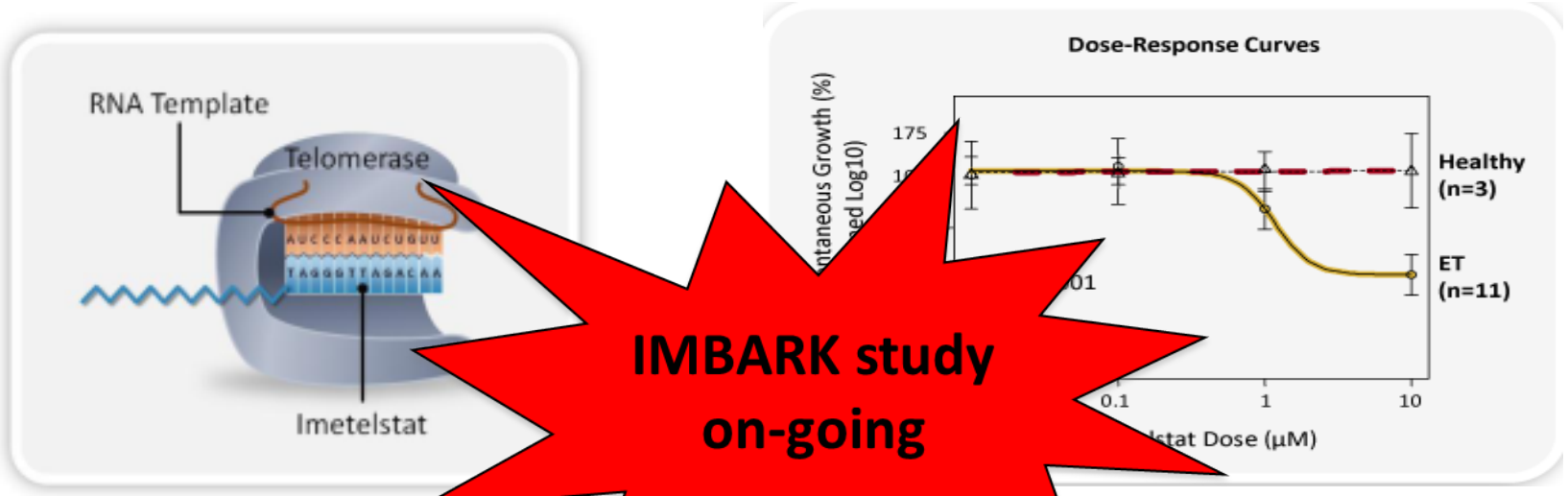


Dysregulated Telomerase Activity in MPN

- Upregulated telomerase activity may be involved in proliferation and replication immortality of neoplastic progenitor cells, and has been shown to occur also in MPN cells



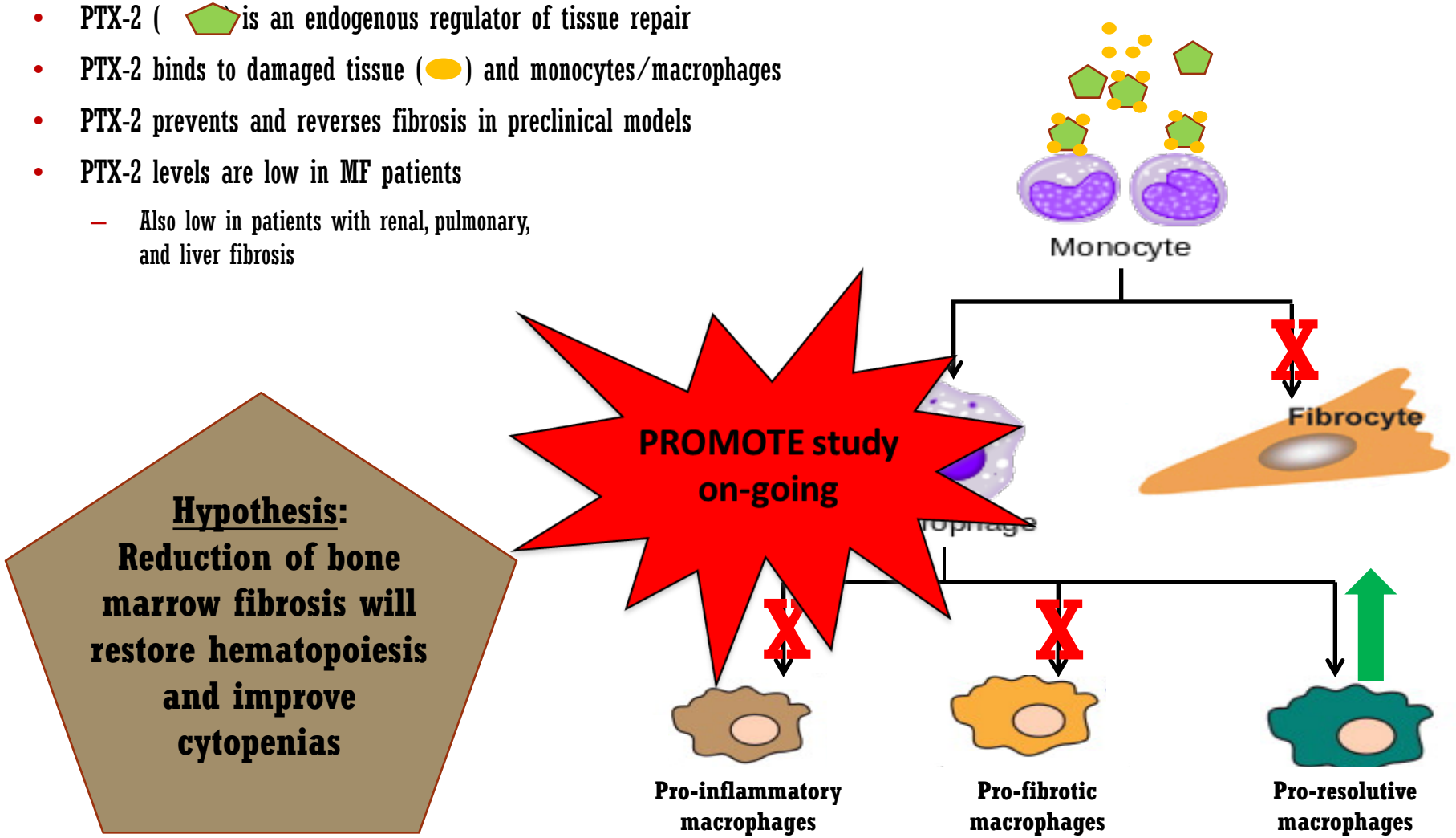
- **IMETELSTAT** is the first telomerase inhibitor in clinical development
- Competitively binds to RNA template of telomerase and inhibits its activity

- **IMETELSTAT** inhibited growth of spontaneous CFU-MK from ET patients
- Did not inhibit cytokine-induced CFU-MK growth from healthy controls

Ruella M, et al. *Exp Hematol*. 2013;41:627-634; Baerlocher GM, et al. *Blood.* 2012;120:abstract 179.

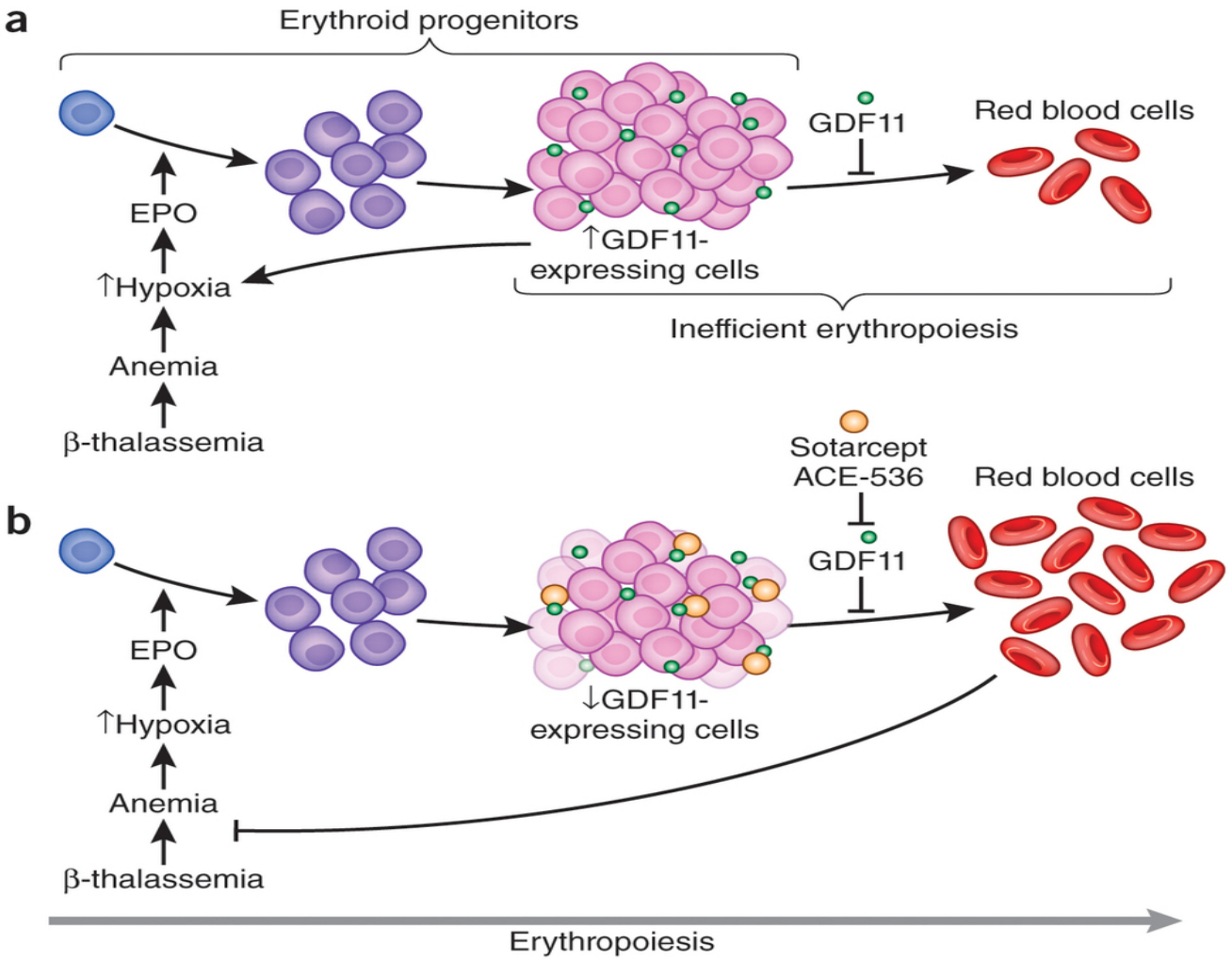
PRM-151: RECOMBINANT HUMAN PENTRAXIN-2 (PTX-2)

- PTX-2 ( ) is an endogenous regulator of tissue repair
- PTX-2 binds to damaged tissue ( ) and monocytes/macrophages
- PTX-2 prevents and reverses fibrosis in preclinical models
- PTX-2 levels are low in MF patients
  - Also low in patients with renal, pulmonary, and liver fibrosis

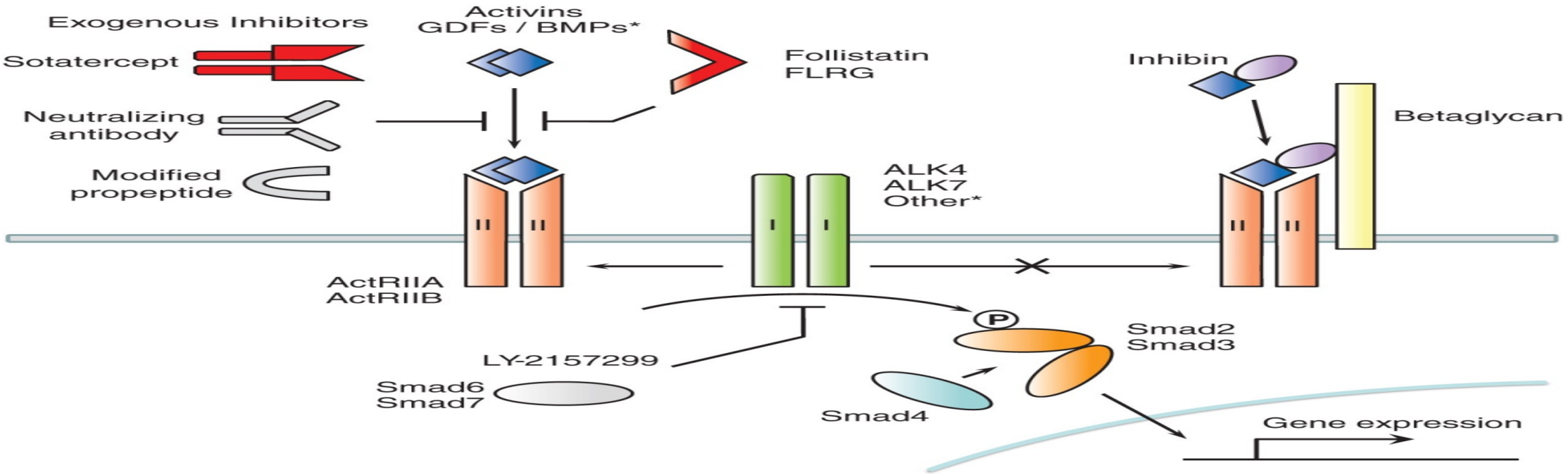


Verstovsek S, et. al. ASH 2014. Abstract

LUSPATERCEPT (ACE-536)

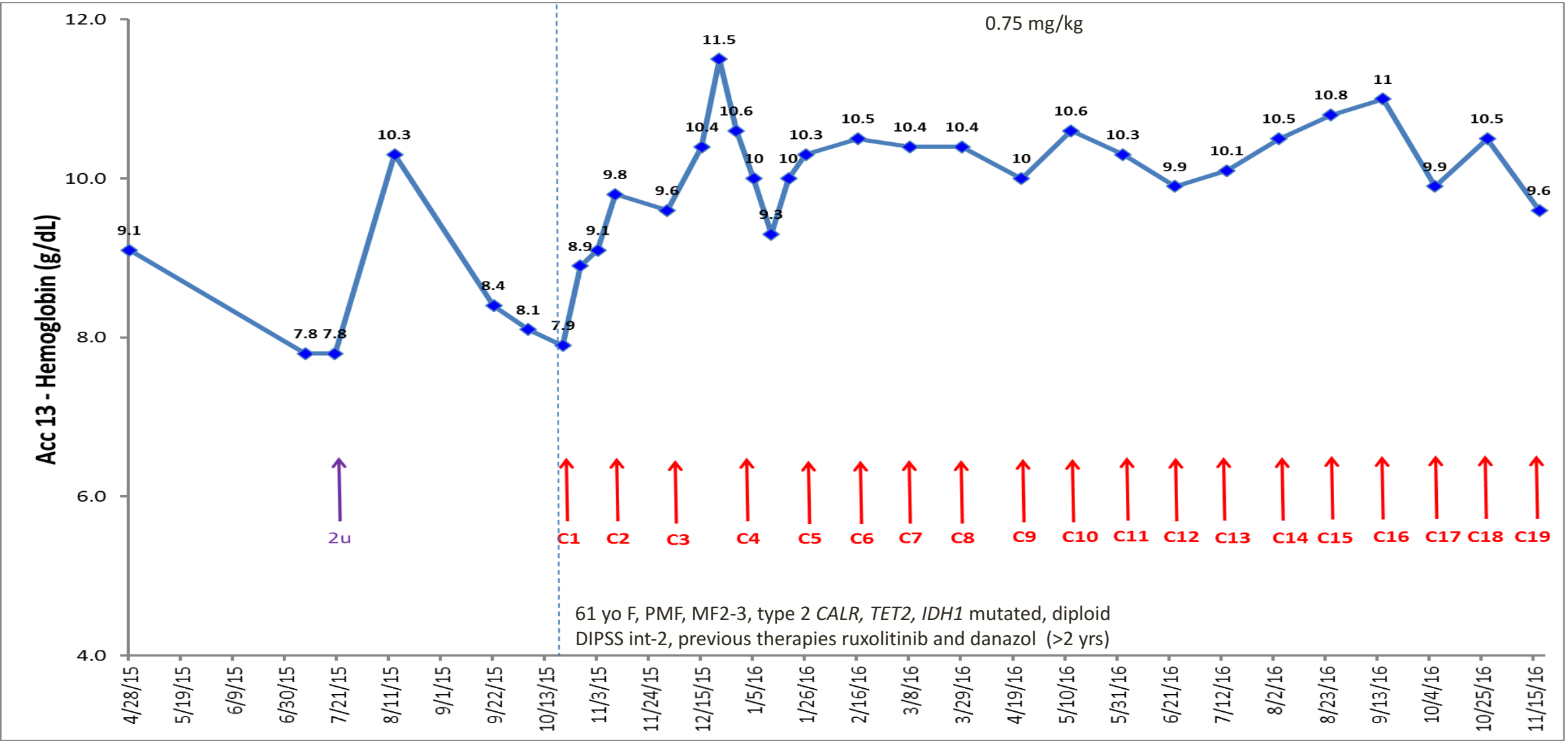


- Sotatercept (ACE-011) a soluble receptor fusion protein (activin receptor type IIA linked to Fc fragment of human IgG1) that "traps" ligands that bind to ActIIRA, relieving blockade of terminal erythropoiesis
- Preclinically in thalassemia, Diamond-Blackfan anemia, hepcidin transgenic mice; clinically in patients with ß-thalassemia and MDS

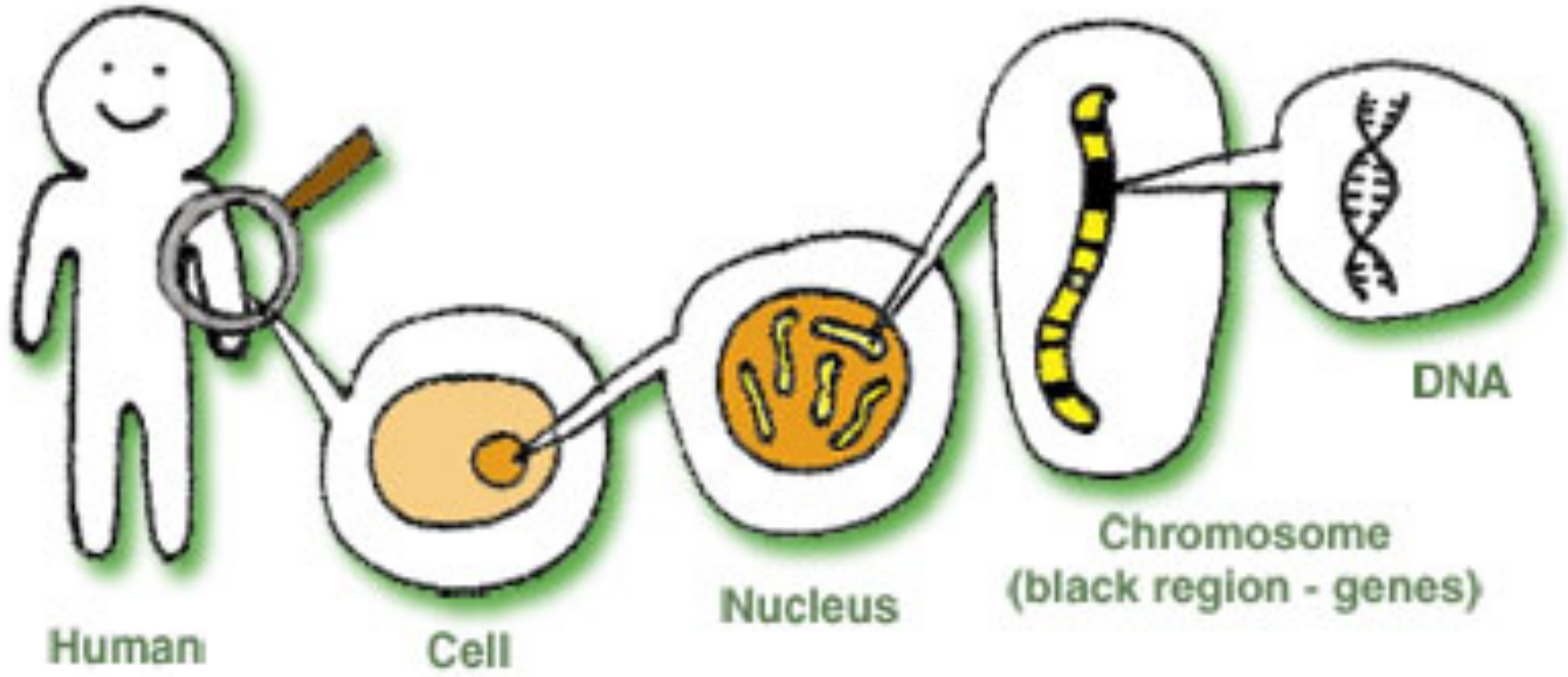


Raftopoulos H et al. Support Care Cancer 2016. Ear J et al. Blood 2015. Langdon JM et al. Am J Hematol 2015.
Dussiot M et al. Nat Med 2014. Carrancio S et al. Br J Haematol 2014. Komrokji R et al. ASH 2014. Iancu-Rubin C et al. Exp Hematol 2013.

Sotatercept in MF



GENETICS: EACH CELL CARRIES A GENOME

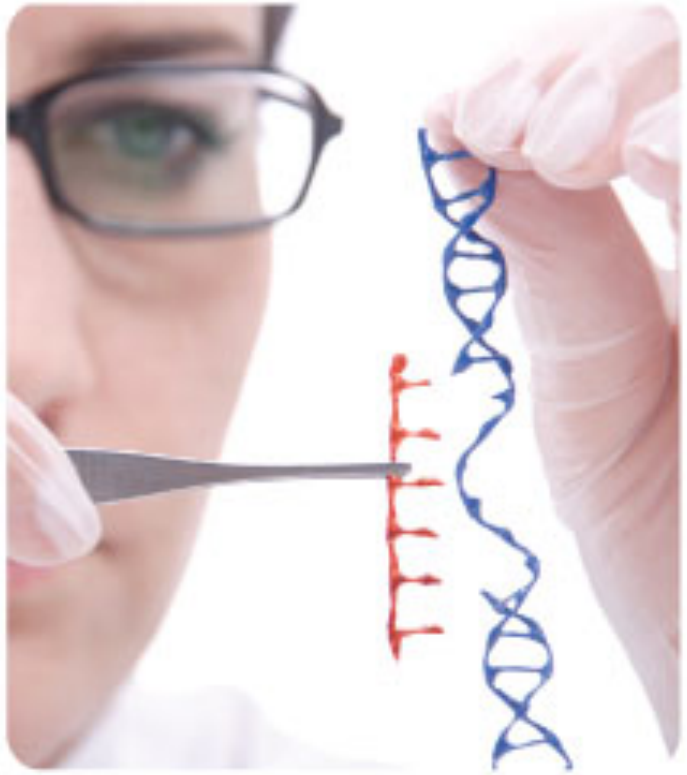


Slide courtesy of Daniel E. Bauer, MD PhD, Dana Faber

- Genome is made up of DNA – which is our genetic code

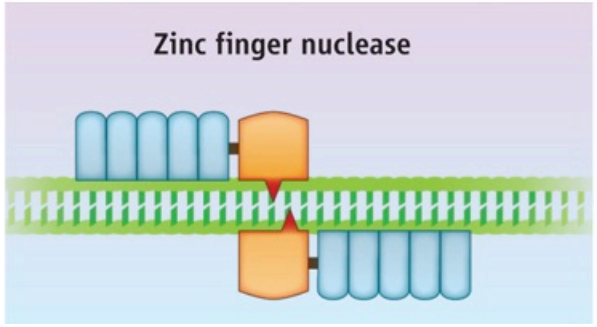
- Acquired mutations are changes in this code:
  - e.g. *JAK2, MPL mutations*

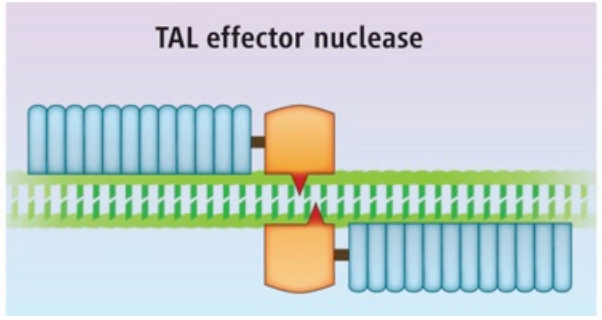
Appealing Concept: Genome Editing

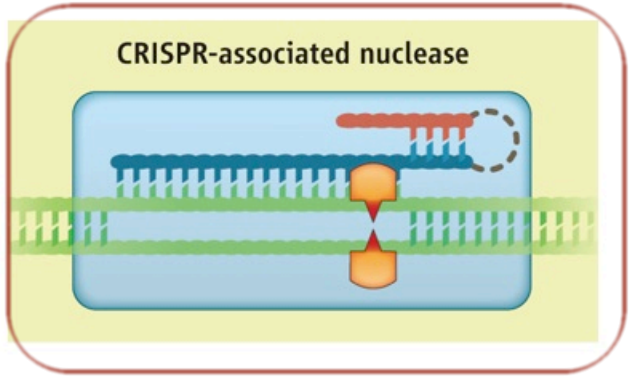


Images courtesy of Daniel E. Bauer, MD PhD, Dana Faber

GENOME EDITING TOOLS ARE SEQUENCE-SPECIFIC NUCLEASES



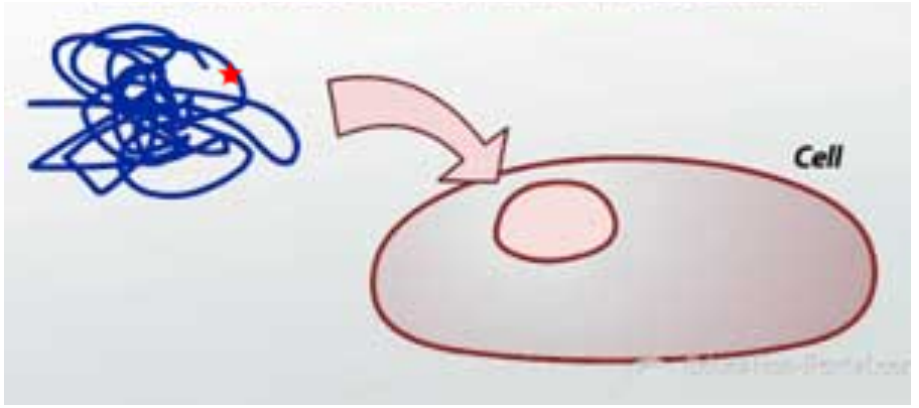




van der Oost. Science (2013) 339:768.

Genome editing tools have two features:

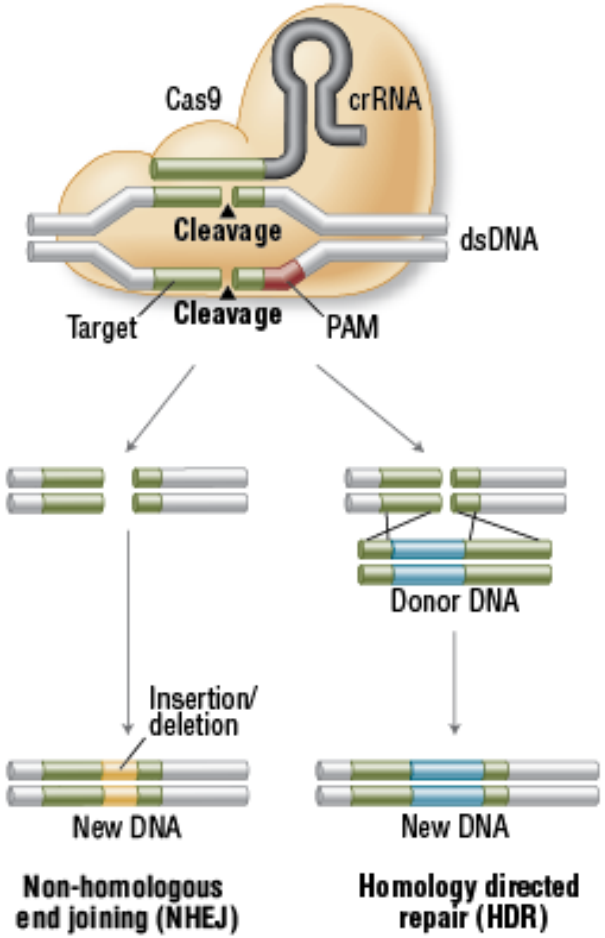
1) Recognize specific DNA sequences (i.e. specific genes or non-coding elements)



2) Cut DNA (“nuclease”), then a scar is left behind

Images courtesy of Daniel E. Bauer, MD PhD, Dana Faber

GENOME EDITING: CLEAVAGE REPAIR CAN EITHER DISRUPT ORIGINAL SEQUENCE OR REPLACE IT WITH A NEW COPY



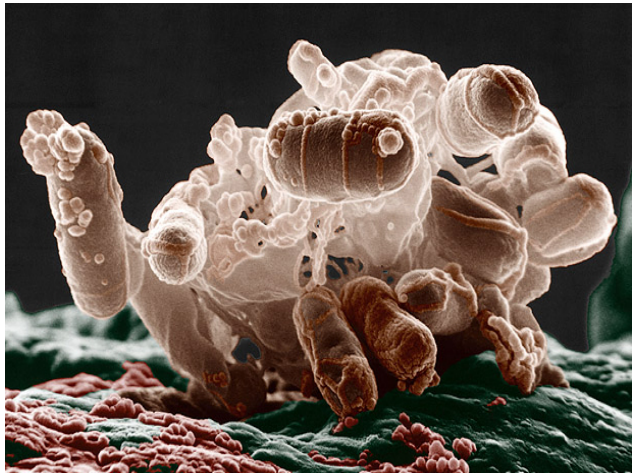
"delete"

"copy and paste"

NEB.com

Images courtesy of Daniel E. Bauer, MD PhD, Dana Faber

Understanding the CRISPR 'Package'



- Bacteria developed mechanisms to combat invading viruses
- 'Stole' part of viral DNA – integrated and duplicated
- Could use transcribed RNA- to mobilise enzymes that found and cut the viral DNA sequence needed for viral reproduction

Pallindrome

Dammit, I'm mad!

DNA Code

G (Guanine)*
A (Adenine)+
T (Thymine)*
C (Cytosine)+

Example of Pallindromic ~~Sequence~~

G A A T T C
C T T A A G

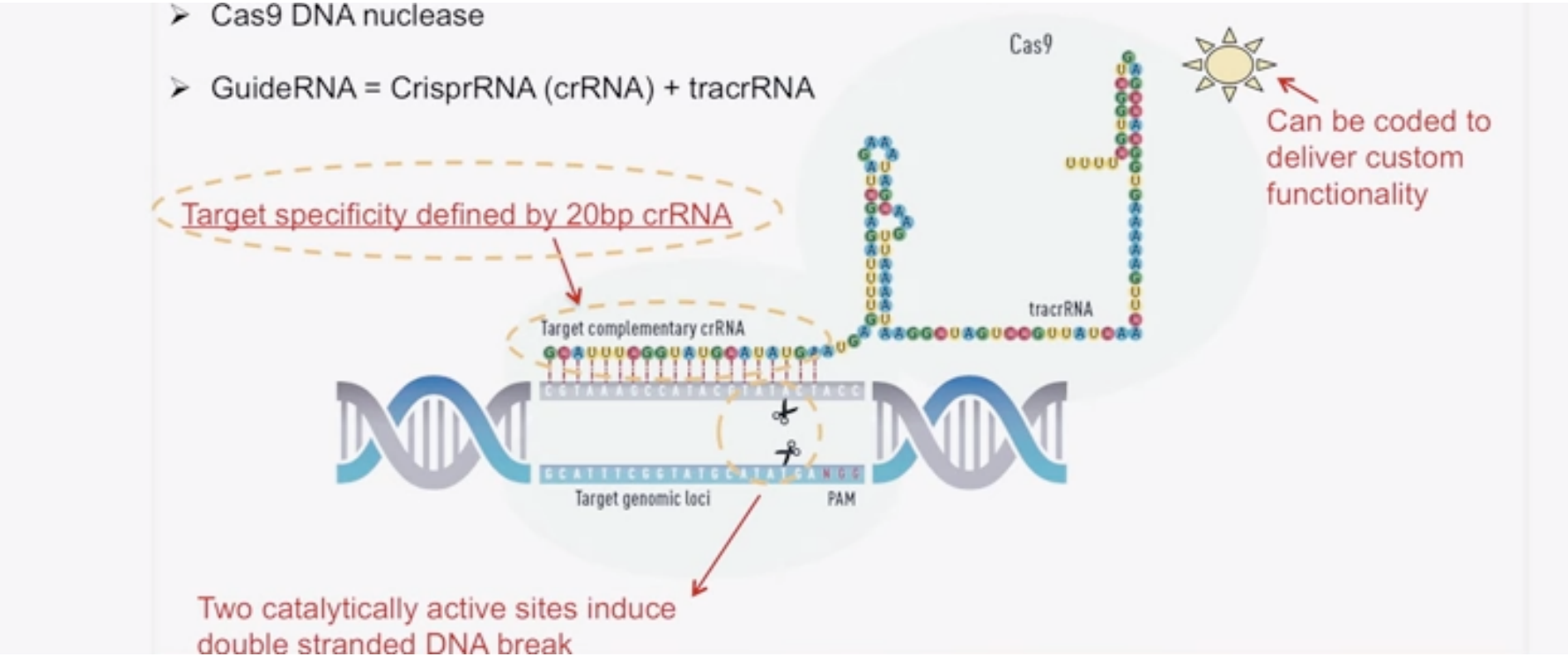
Clustered
Regularly
Interspersed
Short
Palindromic
Repeats.

Cas= CRISPR-ASsociated.

Cas9= the effector

CRISPR: Gene Therapy Finally Coming to MPNs?

- Clustered Regularly Interspaced Short Palindromic Repeat
- Bacterial immune response system leveraged for genome editing



MPNforum Magazine. CRISPR/Cas9: Gene Editing with Precision. www.mpnforum.com/cascade

CRISPR: Gene Therapy Finally Coming to MPNs? Ongoing Exciting research….

George Church, PhD ; Harvard Medical School

"Establishment of isogenic human induced pluripotent stem cell (hiPSC) lines containing CRISPR engineered MPN mutations"

Zhijian Qian, PhD and Wen-Shu Wu, PhD University of Illinois at Chicago

"Correction of JAK2 mutation in myeloproliferative neoplasms by gene editing"

Zhaohui Ye, PhD Johns Hopkins

"Precise Genome Editing for Targeting Malignant Clones in MPNs"

mGEN
MPN Genetics Network

Opinion on CRISPR-Cas Technology for MPN

Exciting and **Novel** Therapy – **BUT-** in its preliminary stages of investigations

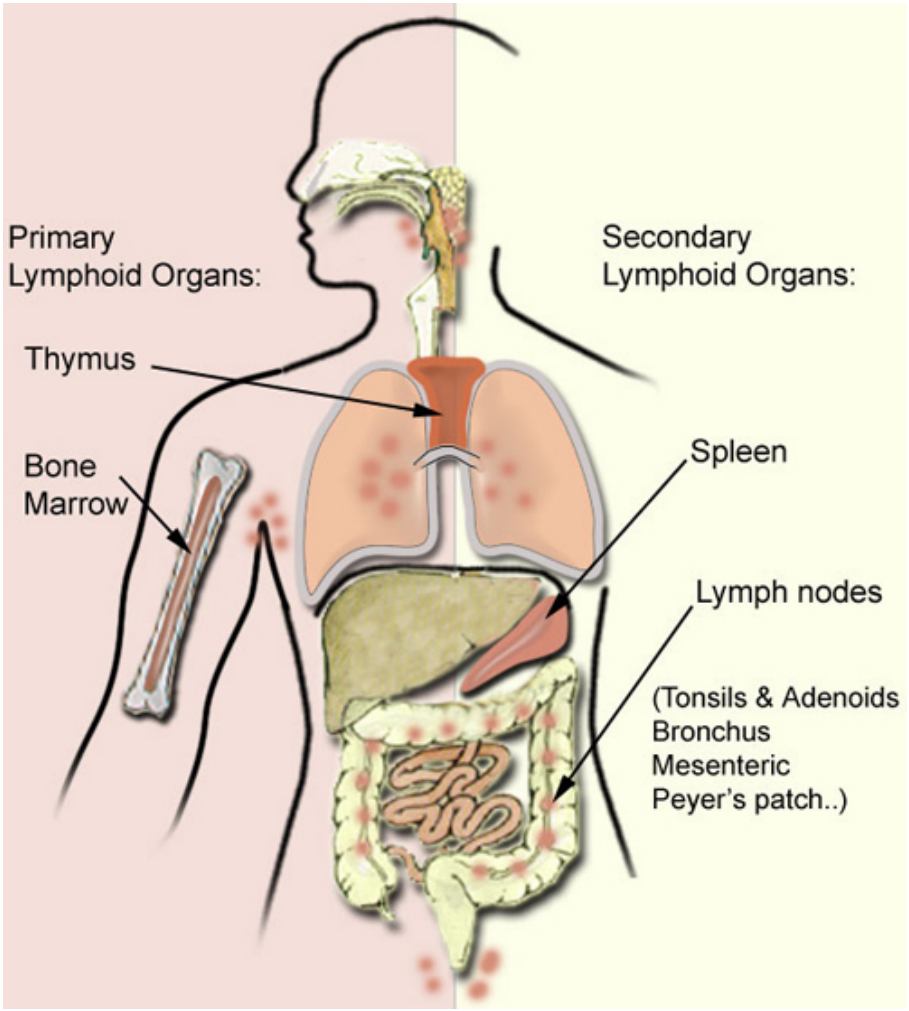
Successfully **Editing** a mutated gene such as *JAK2* or MPL could **IN THEORY** lead to restoration of more normal Function….

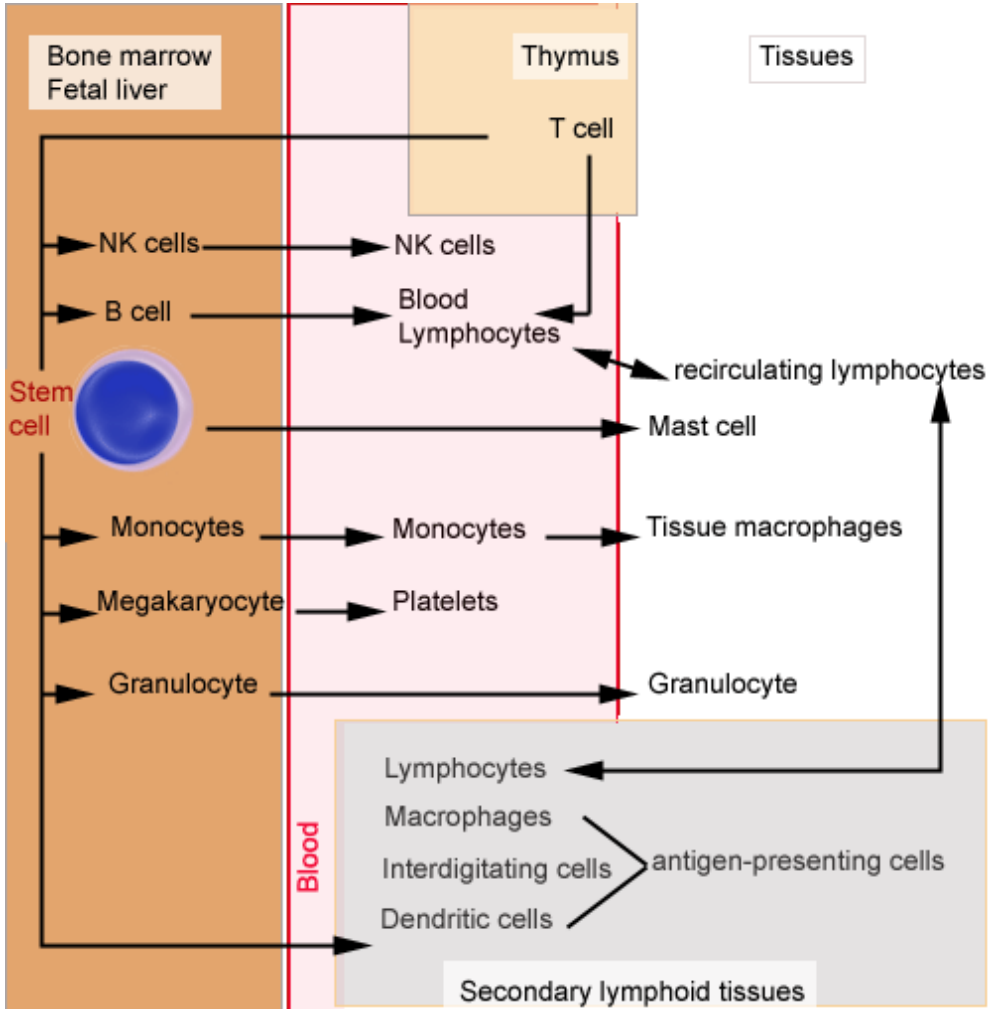
However MANY unanswered questions and safety issues..

- How do we efficiently get a working CRISPR mechanism in specific cells
    - Ex vivo manipulation and reinfusion..
- **Long term** costs and benefits are as yet unclear.
- ? Duration of response and repeat treatments?; Cost

CRISPR still has a long road ahead in development before it can be used to effectively and safely repair – not just disrupt- mutated genes in people.

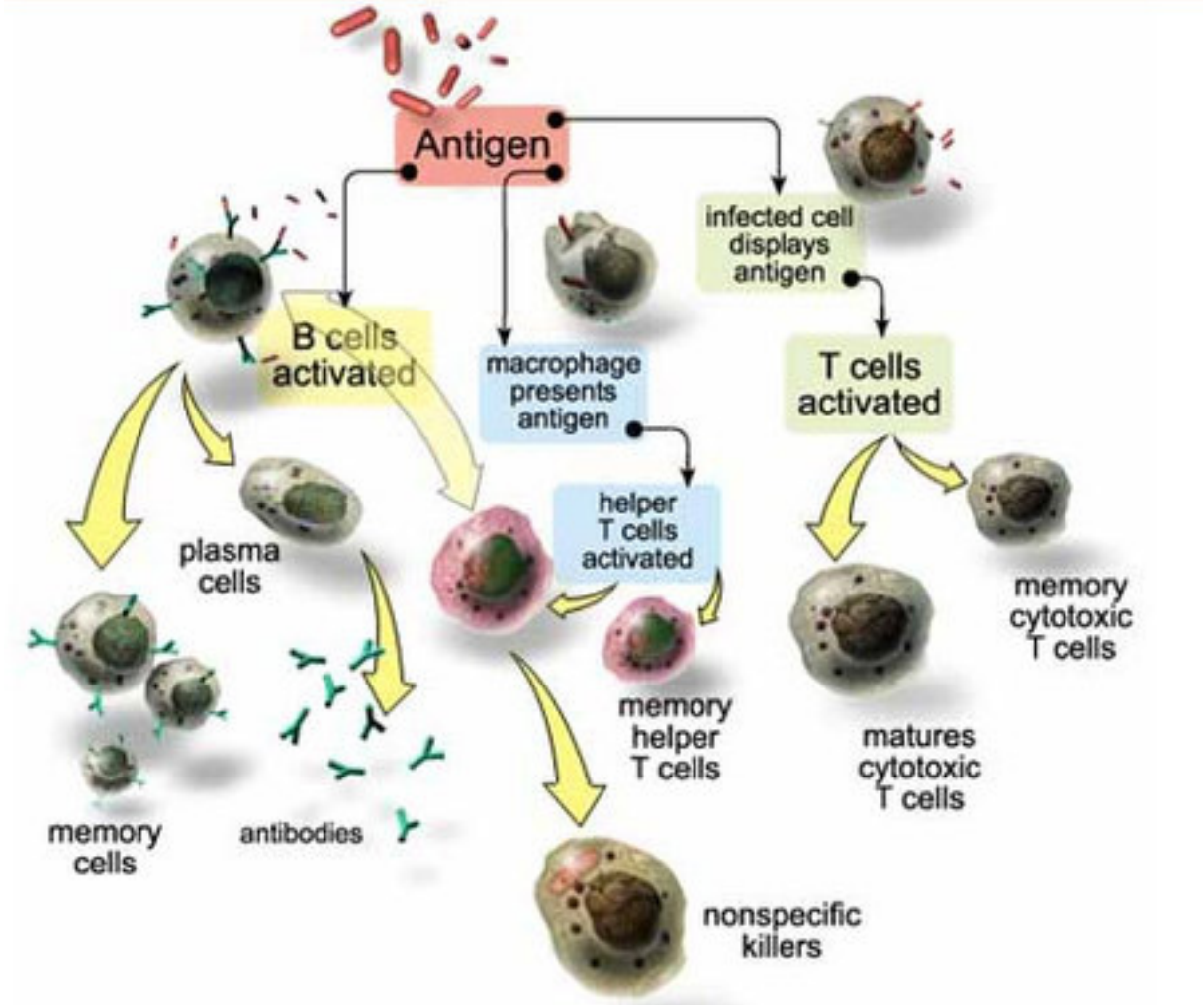
Organisation of the Immune System





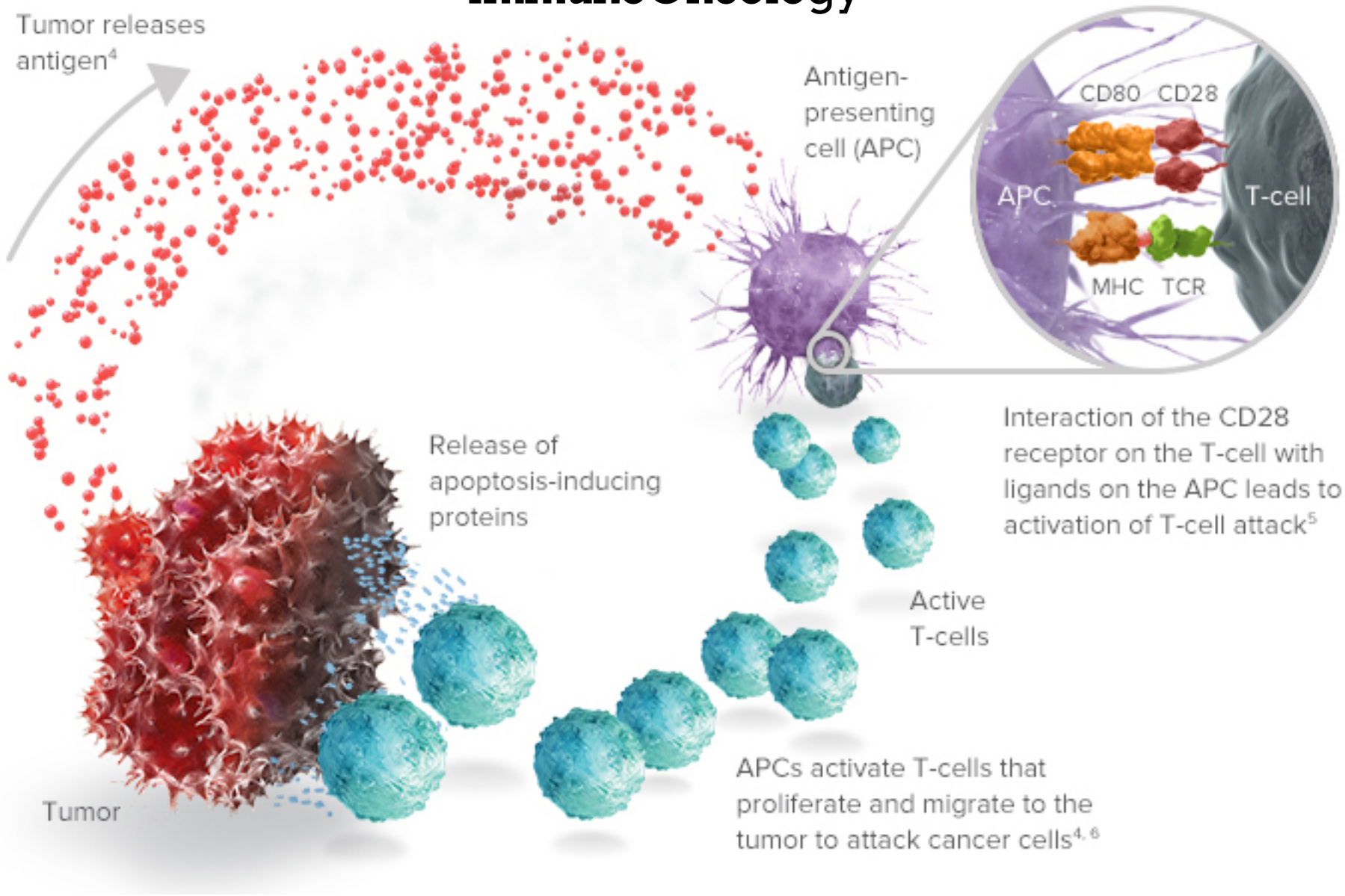
Adapted from McGill Physiology Laboratory Website

Immune system cells



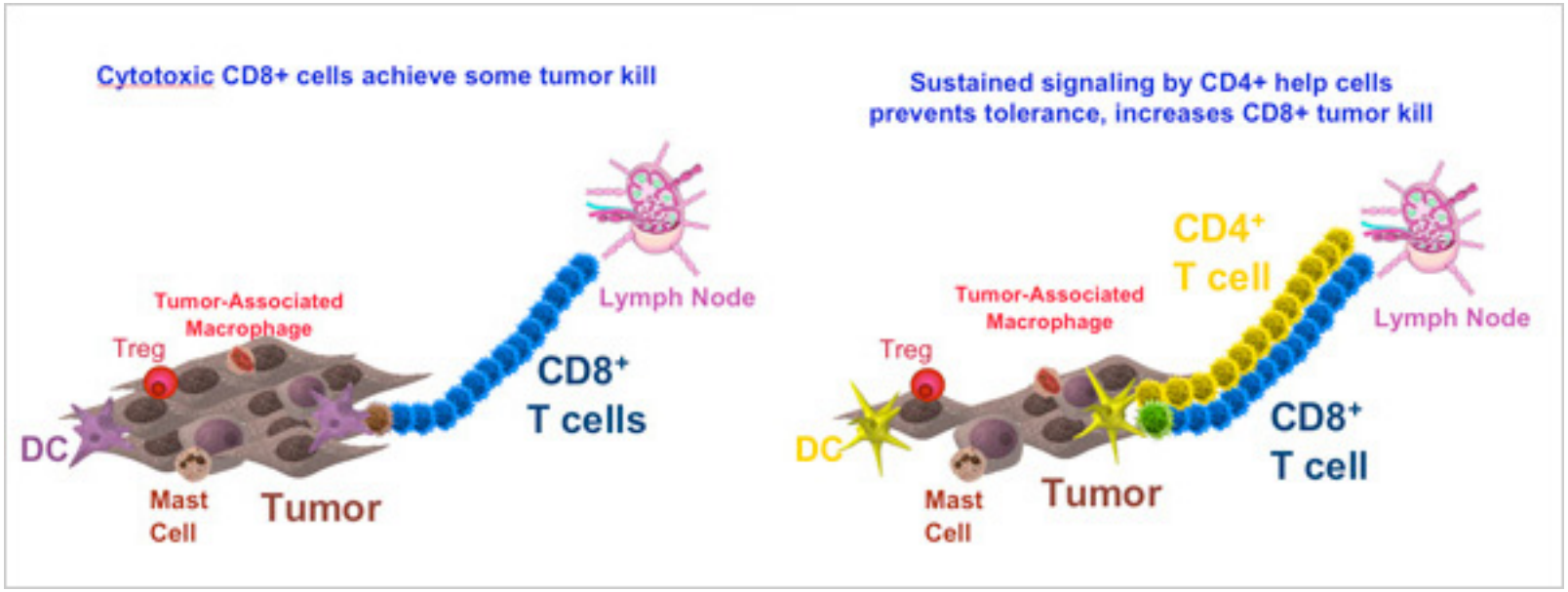
(Source: the Human Immune Response System www.uta.edu/chagas/images/immunSys.jpg)

ImmunoOncology



Multiple effective mechanisms can change this anti-tumour response

ImmunoOncology



Cancer Res. 2009 Jul 21.

T Lymphocytes are very important EFFECTOR cells against Cancer Cells

Multiple effective mechanisms can change this antitumor response

Novel Treatments have been developed that have revolutionised Anti-Cancer Treatment

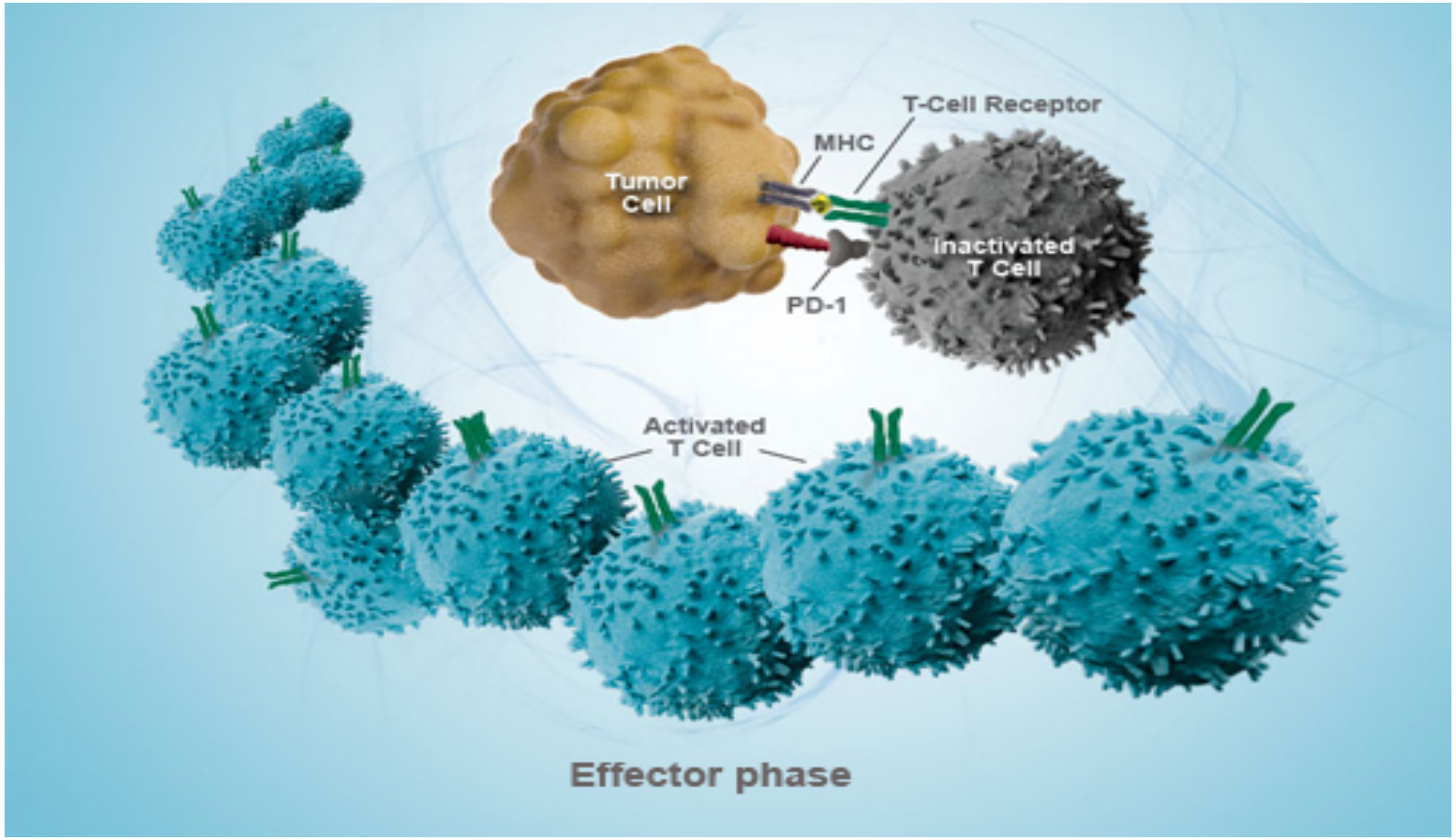
Pivotal Research in Immuno-Oncology is designed to target specific mechanisms in the anti-neoplasm immune response.

This includes the so-called immune checkpoints e.g.

Programmed Cell Death-1 (PD-1)

- Tumour cells often take advantage of these checkpoints to escape detection by the immune system.
- PD-1 (programmed death-1), a protein (receptor) found on the surface of activated T-cells.
- Binding of a protein called PD-L1 to the PD-1 receptor tells the soldiers (i.e., T-cells) to stop fighting- often expressed on the surface of cancer cells where acts as a signal to stop the T-cells' attack

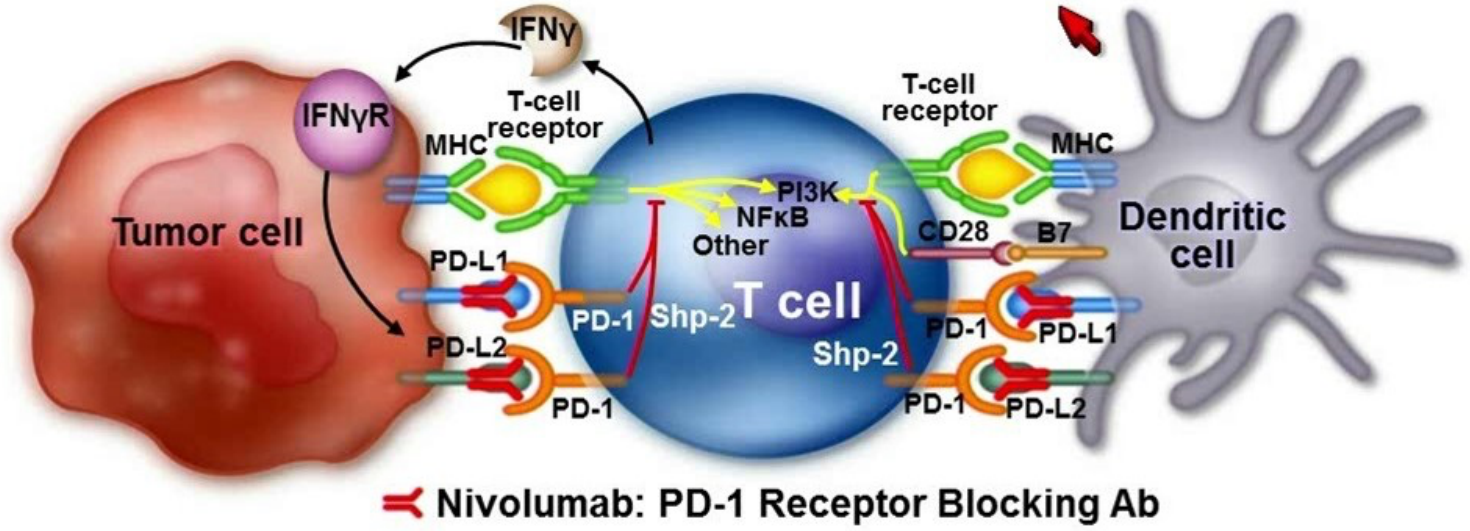
Novel Treatments have been developed that have revolutionised Anti-Cancer Treatment



Immunotherapy drugs such as Nivolumab can block the tumour cell
from deactivating the immune system

Nivolumab Mechanism of Action

- PD-1 expression on tumor-infiltrating lymphocytes is associated with decreased cytokine production and effector function[10]
- Nivolumab binds PD-1 receptors on T cells and disrupts negative signaling triggered by PD-L1/PD-L2 to restore T-cell antitumor function[11–13]



Adapted from www.thepharmacology.com

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

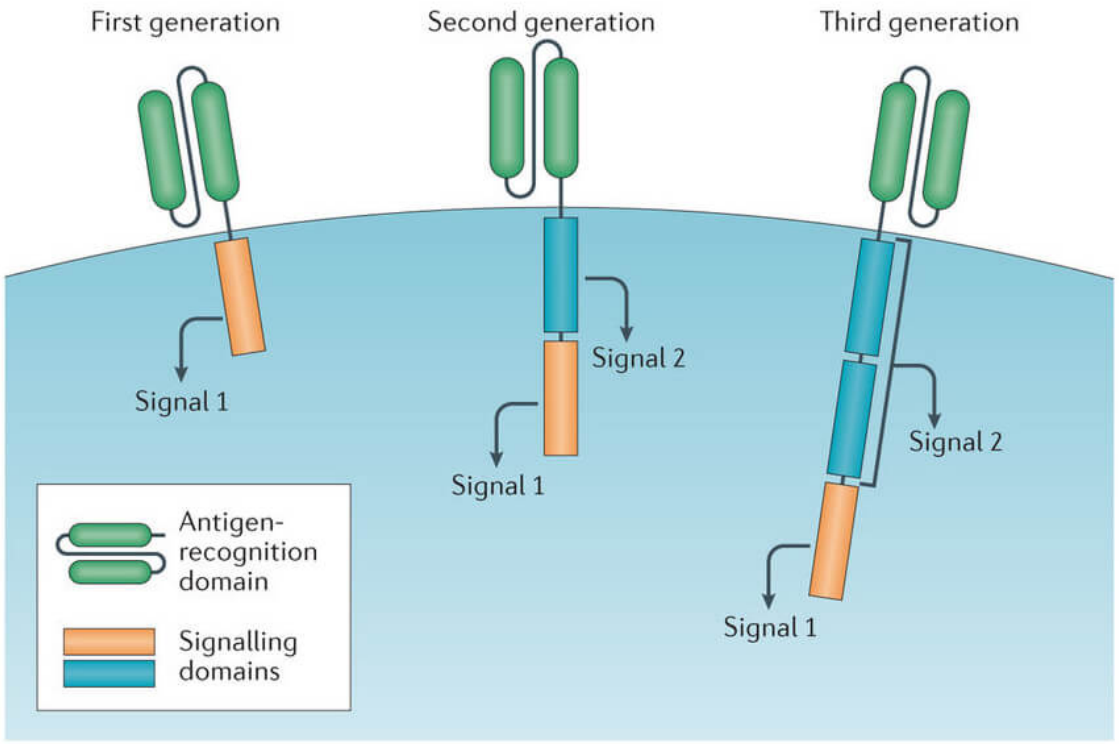
Novel Phase II Trial of Nivolumab in Myelofibrosis

Current Phase II trial for those with Myelofibrosis

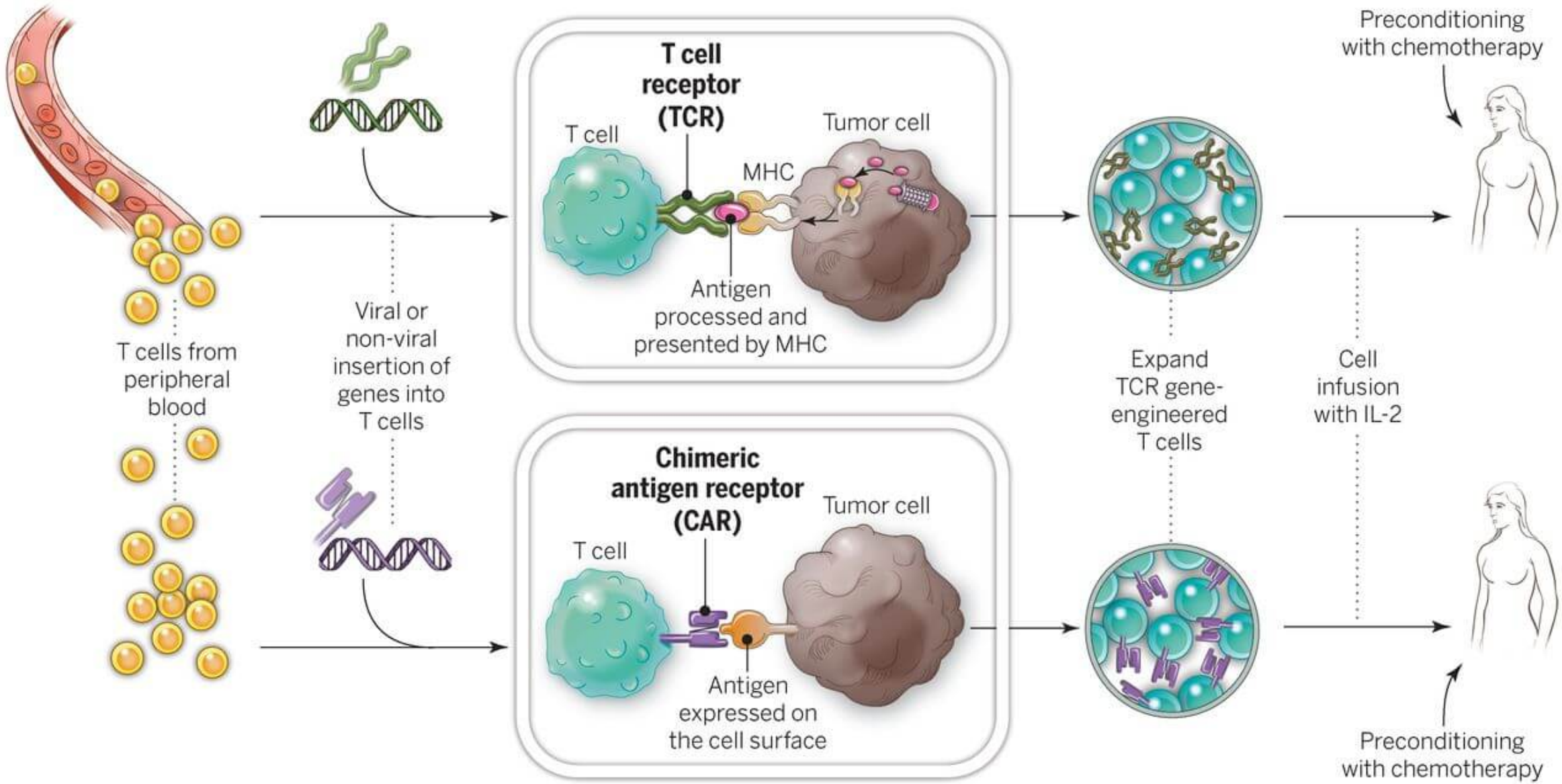
1. Intermediate I,II and High Risk
2. Palpable Spleen
3. Previously failed Ruxolitinib

1. Nivolumab given by an infusion every 2 weeks for a total of 8 doses
2. Then one dose every 3 months
3. Will investigate effects on spleen, symptoms, QOL and blood counts/ marrow
4. Most common Side Effects are fatigue, diarrhoea; muscle aches and nausea.

CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS



Nature Reviews | Clinical Oncology


T cells from peripheral blood
Viral or non-viral insertion of genes into T cells
T cell receptor (TCR)
T cell
Tumor cell
MHC
Antigen processed and presented by MHC
Chimeric antigen receptor (CAR)
T cell
Tumor cell
Antigen expressed on the cell surface
Expand TCR gene-engineered T cells
Cell infusion with IL-2
Preconditioning with chemotherapy
Preconditioning with chemotherapy