The Role of HHLA2 in Non-Small Cell Lung Cancer

Name of student
Off-Topic Candidacy Exam
Date of Exam

Advisor: Name of Advisor

Lung Cancer Statistics

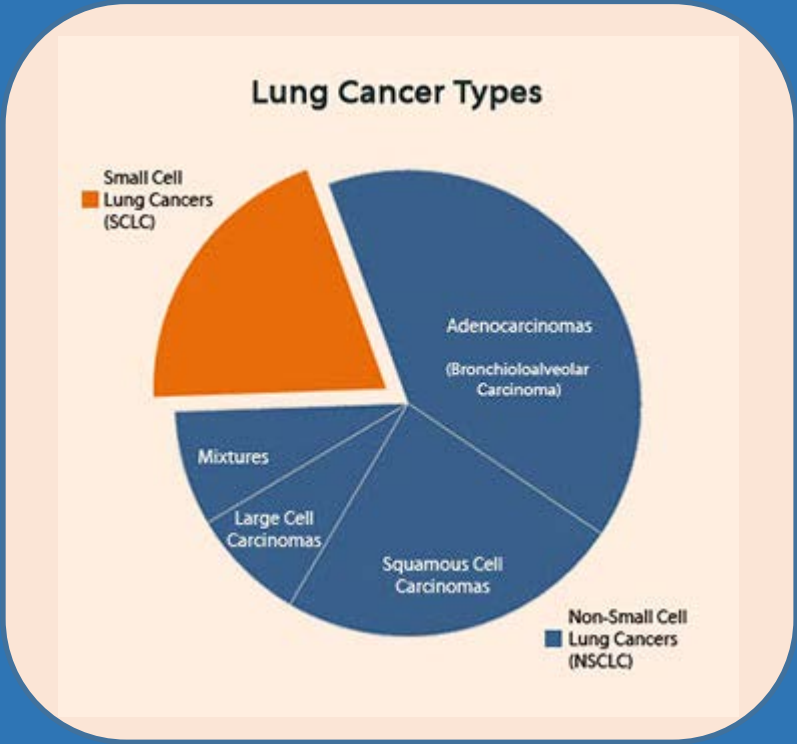
- Lung cancer is the second most common cancer in the USA, with ~200,000 new cases annually.

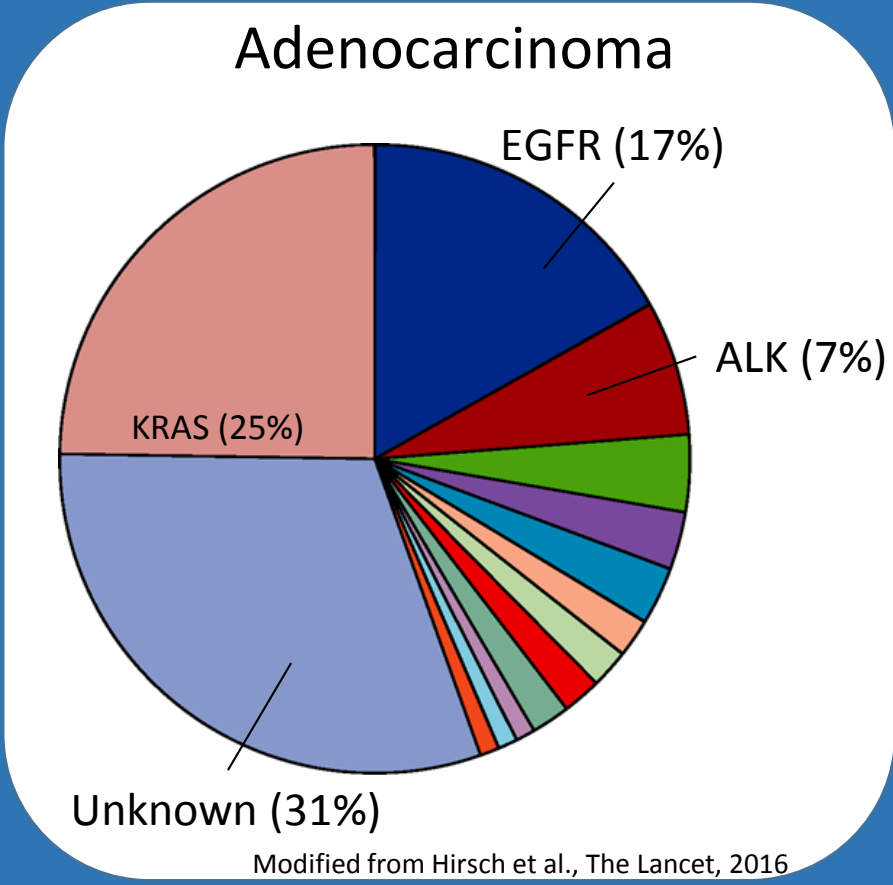
Estimated Deaths

Males				Females		
Lung & bronchus	84,590	27%		Lung & bronchus	71,280	25%
Colon & rectum	27,150	9%		Breast	40,610	14%
Prostate	26,730	8%		Colon & rectum	23,110	8%
Pancreas	22,300	7%		Pancreas	20,790	7%
Liver & intrahepatic bile duct	19,610	6%		Ovary	14,080	5%
Leukemia	14,300	4%		Uterine corpus	10,920	4%
Esophagus	12,720	4%		Leukemia	10,200	4%
Urinary bladder	12,240	4%		Liver & intrahepatic bile duct	9,310	3%
Non-Hodgkin lymphoma	11,450	4%		Non-Hodgkin lymphoma	8,690	3%
Brain & other nervous system	9,620	3%		Brain & other nervous system	7,080	3%
All Sites	**318,420**	**100%**		**All Sites**	**282,500**	**100%**

Siegel et al., CA: A Cancer Journal for Clinicians, 2017

The Biology of Lung Cancer

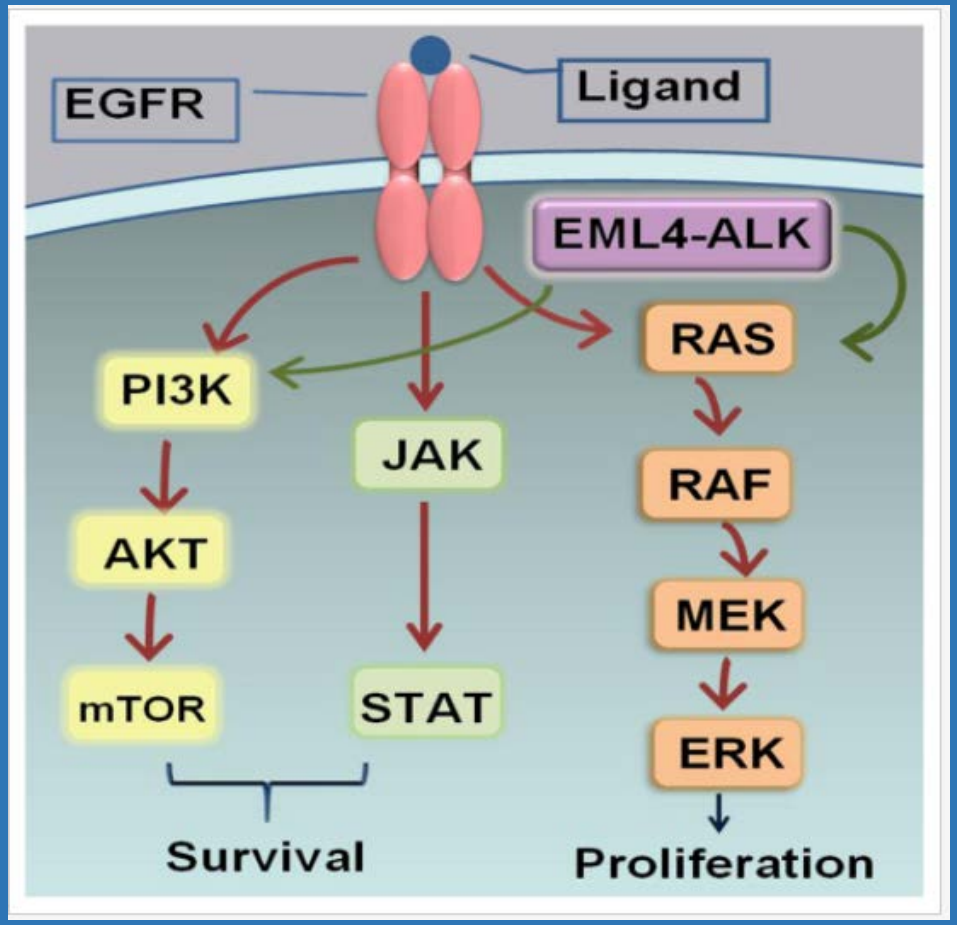




Modified from Hirsch et al., The Lancet, 2016

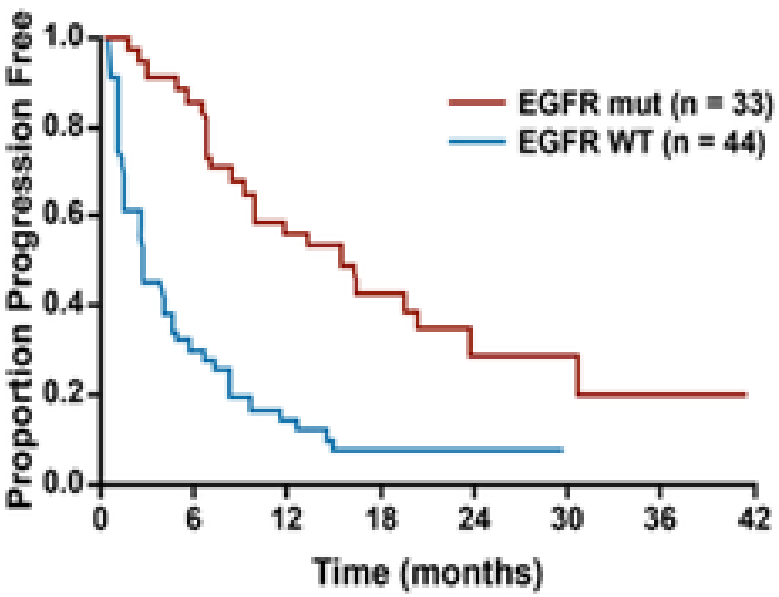
- Understanding histology and molecular landscape of NSCLC provides basis for treatment decisions.

Rationale for Targeting Specific Driving Mutations

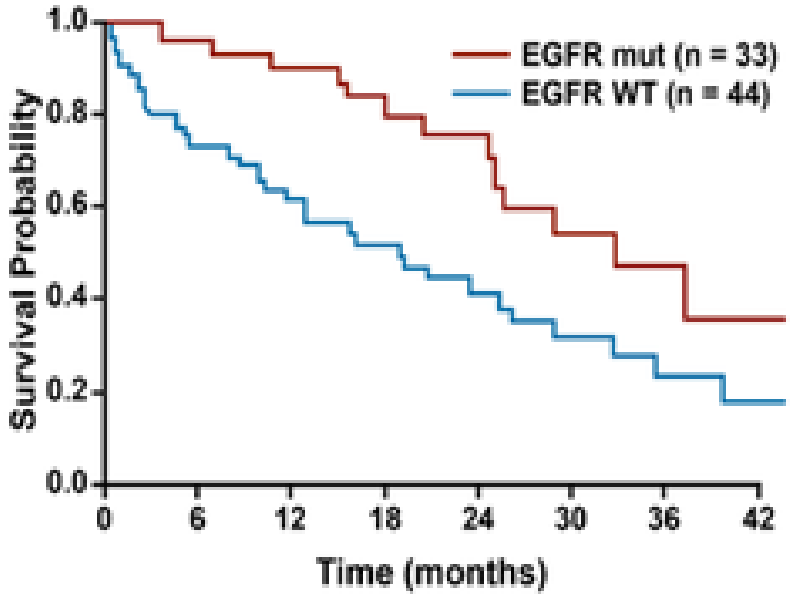


Wu, Int. J. Mol. Sci. 2012

EGFR TKIs Show More Benefit in EGFR Mutant Patients



EGFR mut : 15.7 (8.6-20.4)
EGFR WT: 2.7 (1.4-4.4)
P < .0001



EGFR mut : 31.3 (23.8-42.8)
EGFR WT: 18.1 (9.5-25.0)
P = .0093

RR **EGFR mut: 67%; EGFR WT: 9%; *P* < .0001**

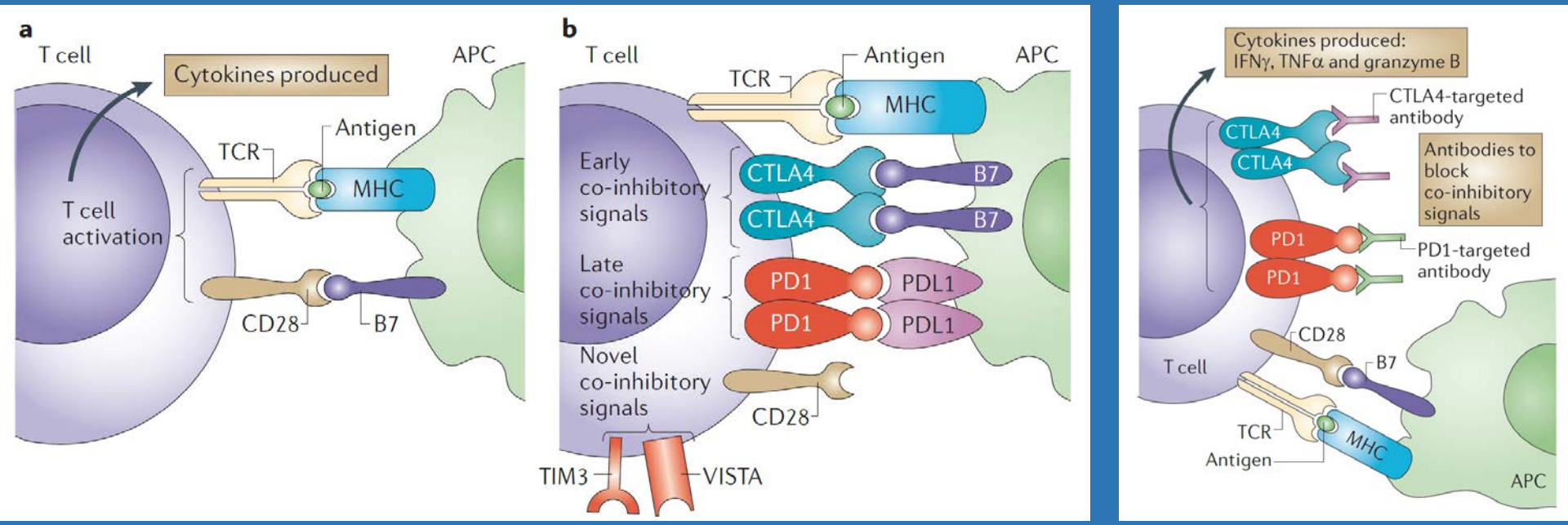
Mut = mutant; WT = wild type

Janne PA, et al. *J Clin Oncol*. 2010;28:7503.

Issues of Specific TKIs

- Only patients who harbor the specific mutations can benefit from TKIs. (He et al., Med Sci Monit. 2016)
- Resistance to TKIs often occur, leading to relapse. (Reviewed in Neel, Nature, 2017)
  - 70% of patients who harbor EGFR mutations will have a prolonged PFS of about one year, then gain resistance and relapse.

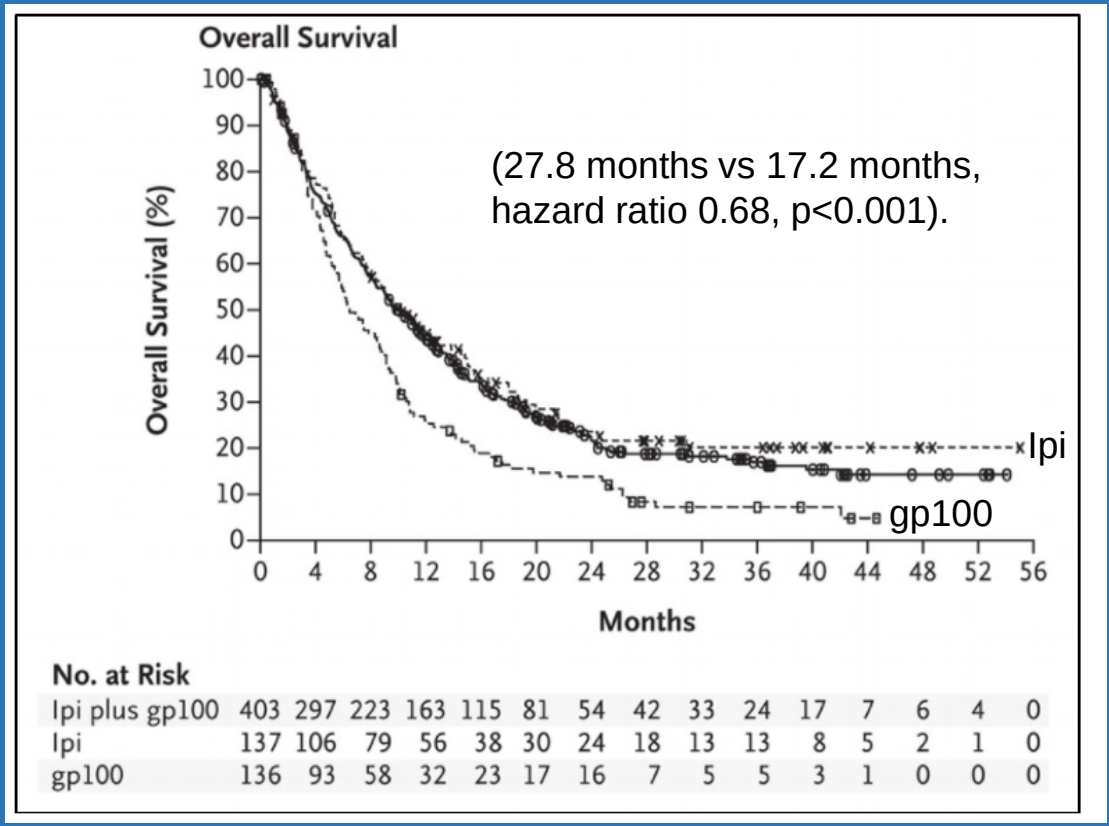
Immunotherapy Can Reverse Tumor Immune Escape



Sharma et al., NRC, 2011

Blocking CTLA-4 is Therapeutically Efficacious in Melanoma

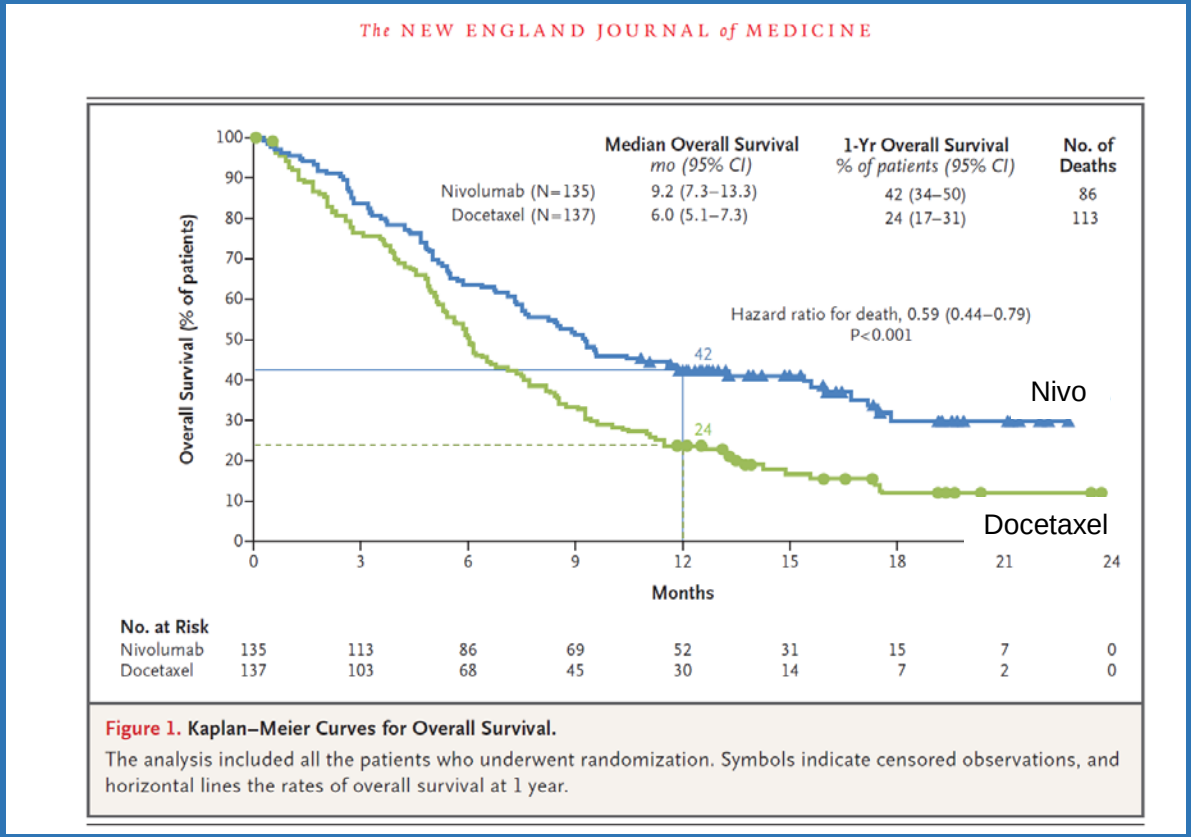
- The CTLA-4 blocking antibody was FDA approved for treatment of metastatic melanoma in 2011.



Hodi et al., NEJM, 2010

Blocking PD-1 is Therapeutically Efficacious in Lung Cancer

- PD-1 blocking antibody, Nivolumab was FDA approved for treatment of advanced lung cancer in 2015.



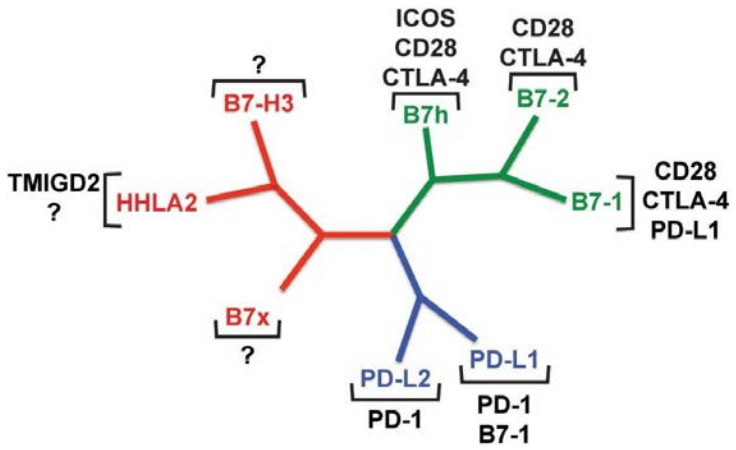
Brahmer et al., NEJM, 2015

Patients Treated with Both Specific TKIs and Immunotherapy Do Relapse

- There is an unmet need of alternative therapies for patients who relapse from initially beneficial drugs.

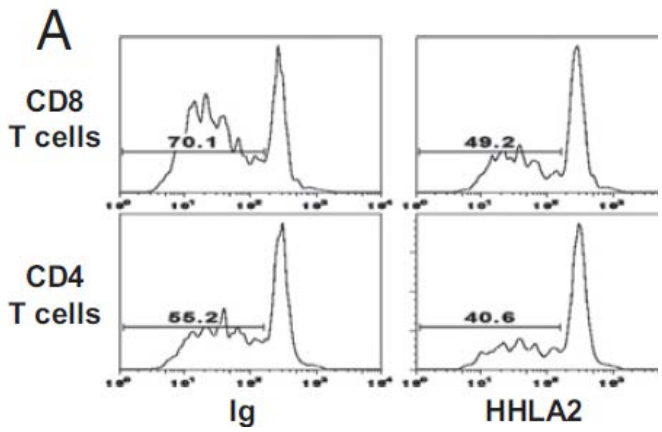
❖ New Targets
❖ Combination Therapies

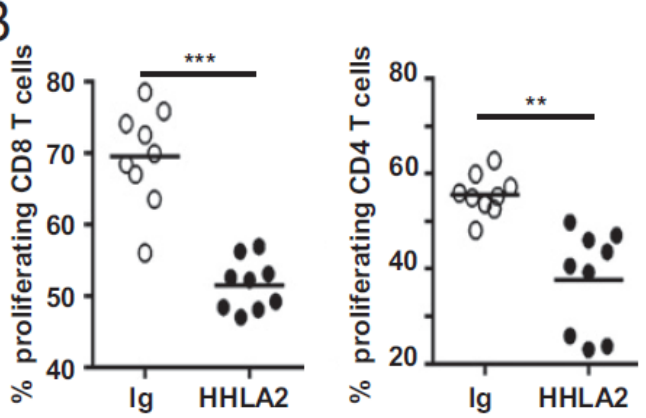
HHLA2 is a Recently Discovered T-cell Co-inhibitory Molecule



Janakarim et al., 2015, CCR

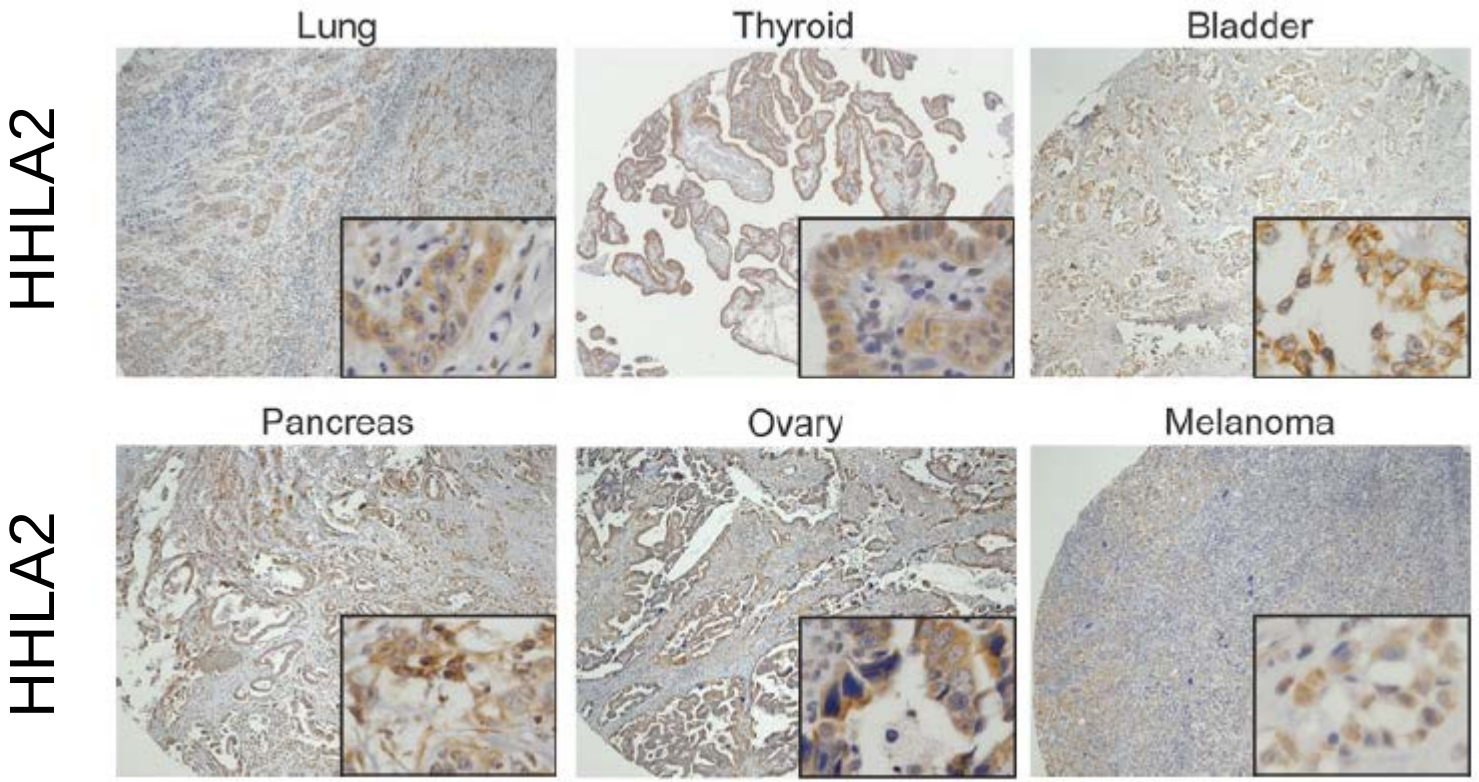
T-cells from PBMCs cultured with an HHLA2 peptide led to lower proliferation of both CD4 and CD8 T-cells compared to the control peptide.





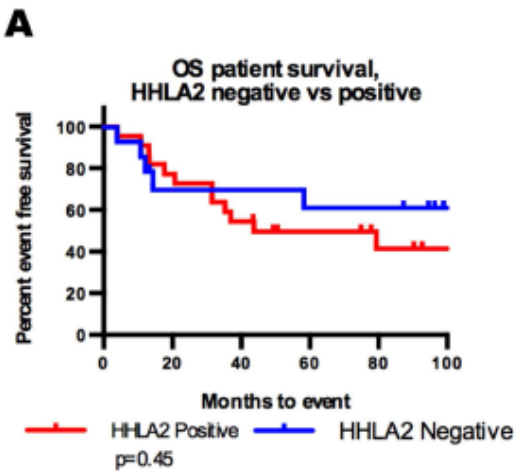
Zhao et al., 2013, PNAS

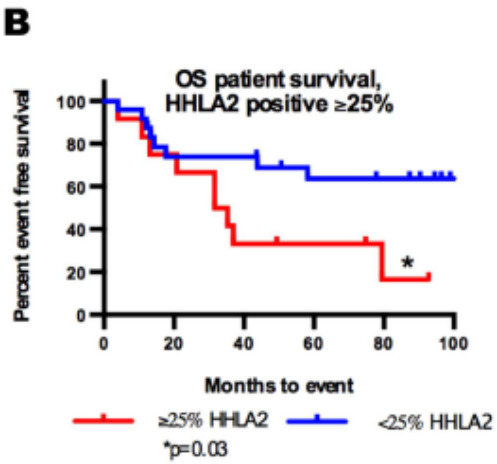
HHLA2 Is Expressed in Many Different Cancers

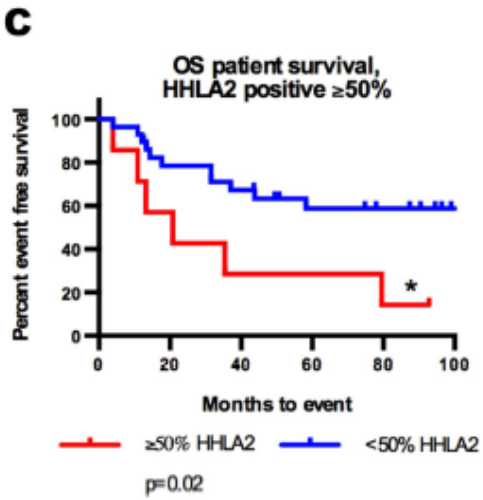


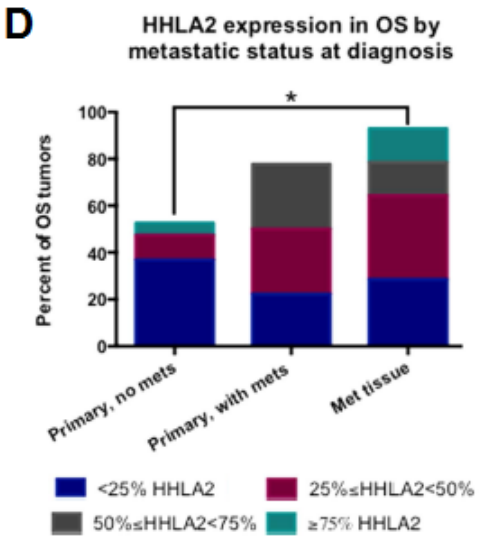
Janakiram et al., 2015, CCR

HHLA2 is Associated with Poor Survival and Metastasis in Osteosarcoma









- A high expression level compared to a low expression level of HHLA2 is associated with a lower rate of survival in osteosarcoma.
- A higher expression rate of HHLA2 is also associated with metastasis in osteosarcoma.

Koirala, 2016, Sci Rep

HHLA2 is Widely Expressed in Lung Cancer

Parameter	Discovery cohort ($n = 392$) HHLA2 Negative	HHLA2 Positive	*P*
Age, year	67.9	67.5	0.78
Gender			0.60
Female ($n = 215$)	78 (36%)	137 (64%)	
Male ($n = 141$)	55 (39%)	86 (61%)	
Histology			<0.0001
Adeno ($n = 290$)	91 (31%)	199 (69%)	
Squam ($n = 31$)	20 (65%)	11 (35%)	
Large ($n = 18$)	16 (89%)	2 (11%)	
Stage			0.09
I ($n = 252$)	85 (34%)	167 (66%)	
II ($n = 47$)	23 (49%)	24 (51%)	
III ($n = 35$)	10 (29%)	25 (71%)	
Mutation status			0.04
EGFR ($n = 41$)	10 (24%)	31 (76%)	
KRAS ($n = 62$)	23 (37%)	39 (63%)	
WT/WT ($n = 91$)	43 (47%)	48 (53%)	

Parameter	Validation cohort ($n = 287$) HHLA2 Negative	HHLA2 Positive	*P*
Age, year	70.5	70	0.47
Gender			0.09
Female ($n = 180$)	46 (26%)	134 (74%)	
Male ($n = 80$)	29 (36%)	51 (64%)	
Histology			0.92
Adeno ($n = 186$)	58 (31%)	128 (69%)	
Squam ($n = 29$)	8 (27%)	21 (73%)	
Large ($n = 3$)	1 (33%)	2 (67%)	
Stage			0.39
I ($n = 157$)	43 (27%)	114 (73%)	
II ($n = 39$)	15 (38%)	24 (61%)	
III ($n = 22$)	7 (31%)	15 (69%)	
Mutation status			0.01
EGFR($n = 44$)	5 (11%)	39 (89%)	
KRAS ($n = 66$)	24 (36%)	42 (64%)	
WT/WT ($n = 88$)	27 (31%)	61 (69%)	

Cheng et al, 2017, CCR

Central Hypothesis

Targeting HHLA2 in NSCLCs will be effective for killing cancer cells through a cytotoxic T-cell mediated mechanism of action.

Specific Aims

Aim 1: Analyze the role of HHLA2 on T-cells within the tumor microenvironment.

Aim 2: Determine the therapeutic efficacy of targeting HHLA2 in NSCLC.

Specific Aim 1

Analyze the role of HHLA2 on T-cells within the tumor microenvironment.

1. Analyze the T-cells of the TME in NSCLC based on HHLA2 expression.
2. Examine the changes of T-cell sub-populations when HHLA2 is blocked within the TME.
3. Investigate the activation of tumor infiltrating lymphocytes (TILs) or splenocytes of HHLA2 expressing NSCLC bearing mice.

Specific Aim 2

Determine the therapeutic efficacy of targeting HHLA2 in NSCLC.

1. Test the therapeutic effect of a blocking HHLA2 in NSCLC.
2. Investigate the therapeutic effect of targeting HHLA2 and PD-L1 *in vivo*.
3. Inspect therapeutic effectiveness of blocking EGFR and HHLA2 in *EGFR* mutant NSCLC *in vivo*.

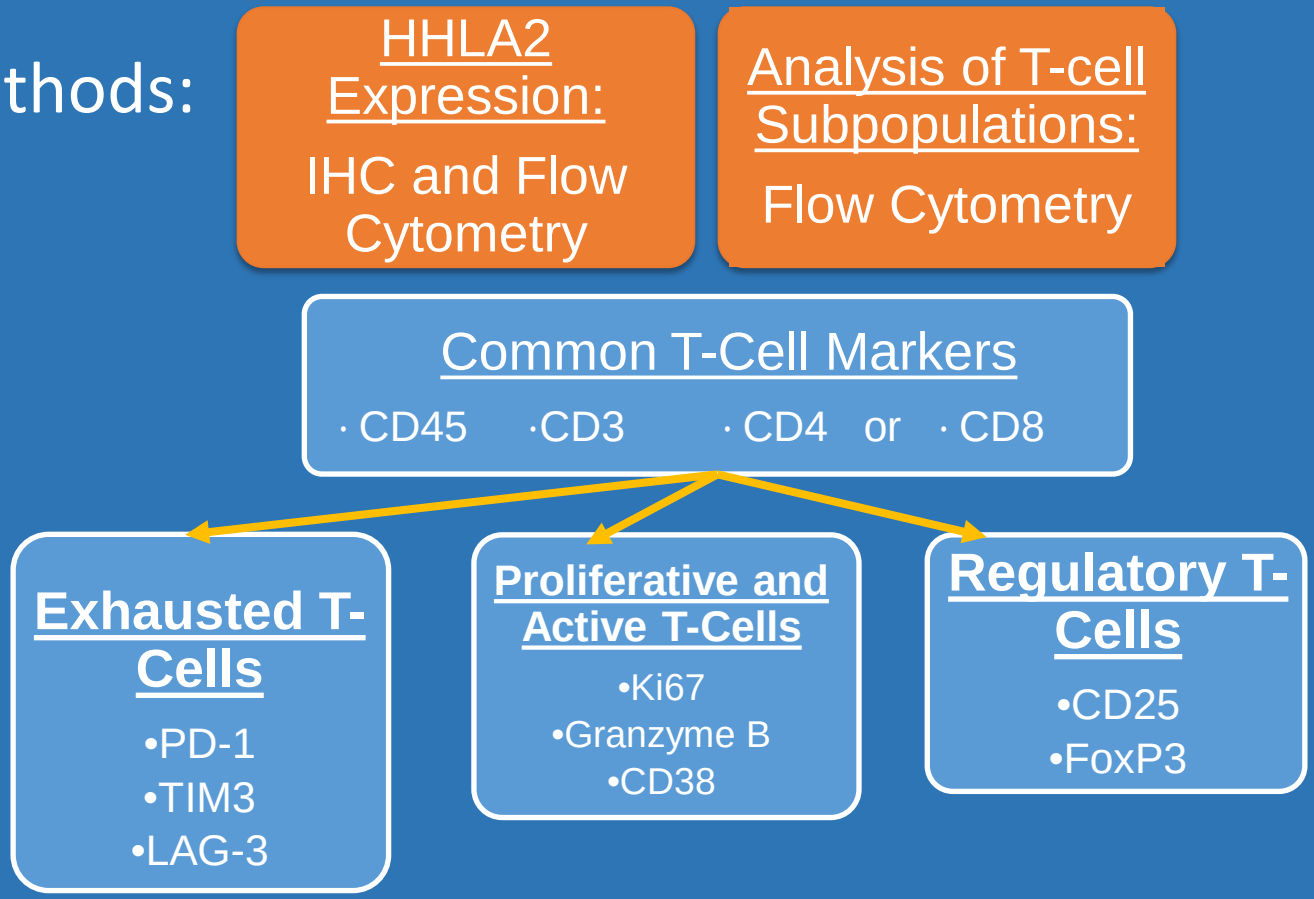
Human Lung Cancer Models

- NSCLC tumor biopsies will be collected from the MD Anderson Department of Thoracic Head and Neck Medical Oncology.

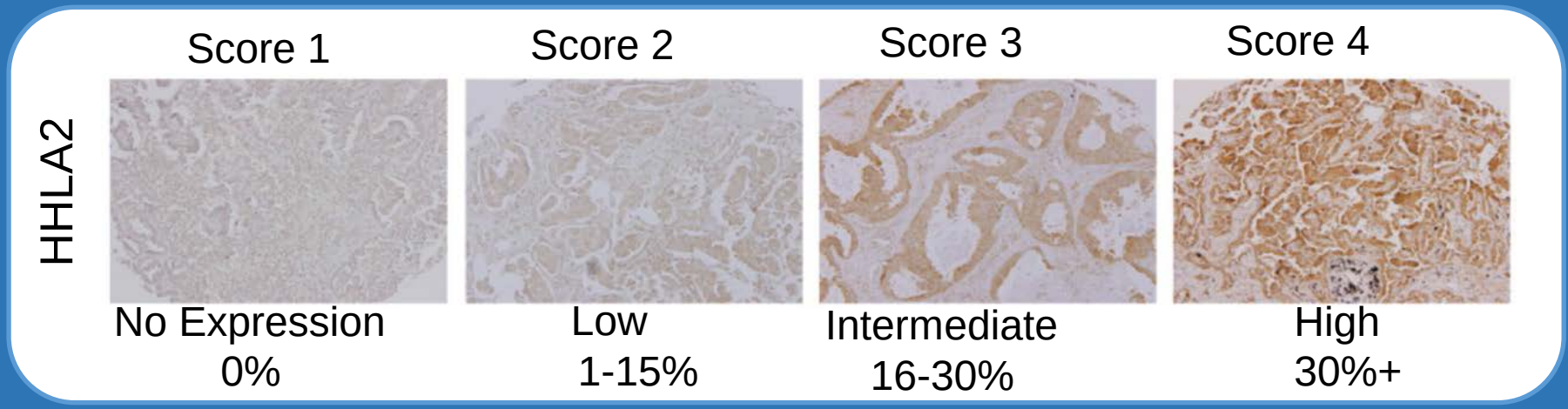
- The Biopsy will be split into three portions
  1. IHC of HHLA2
  2. Flow cytometry of fresh biopsy to analyze T-cell subpopulations of the TME
  3. Development of lung cancer cell lines and matched T-cells

Aim 1.1: Analyze the T-cells of the TME in NSCLC based on HHLA2 expression.

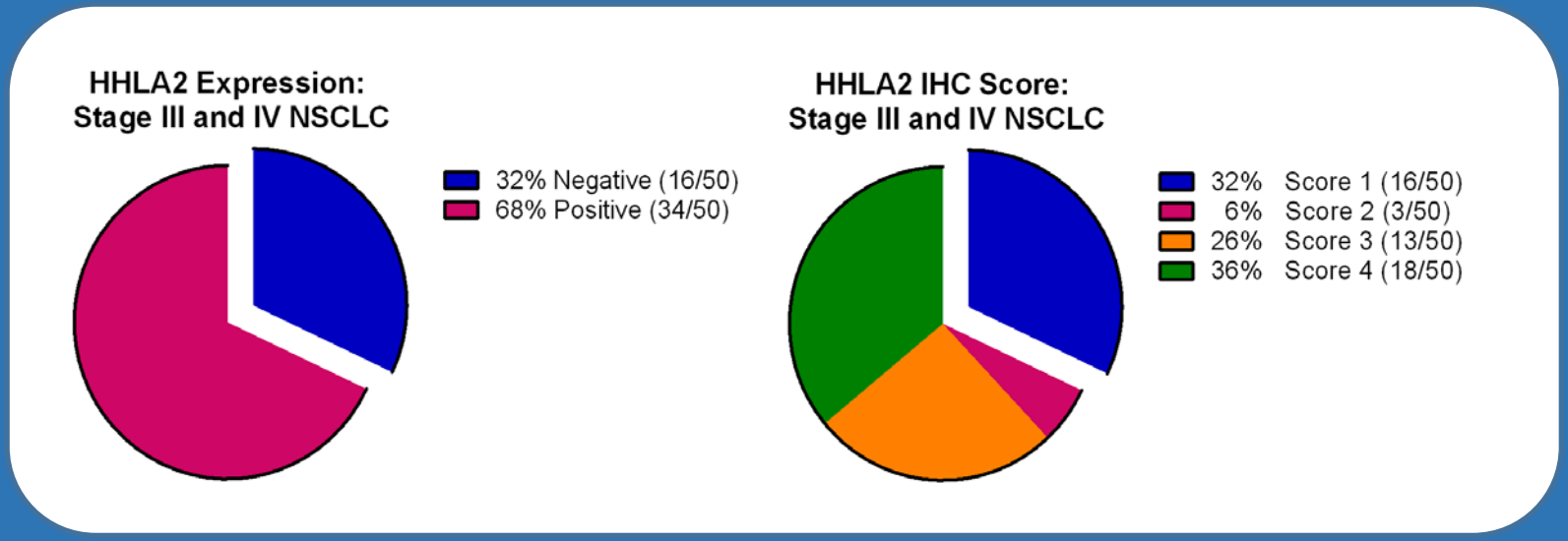
- Materials: Lung Cancer Biopsies
  Human NSCLC Cells and Matched T-Cells
- Methods:



Aim 1.1: Expected Results



Acquired from Chiou et al., Scientific Reports, 2017



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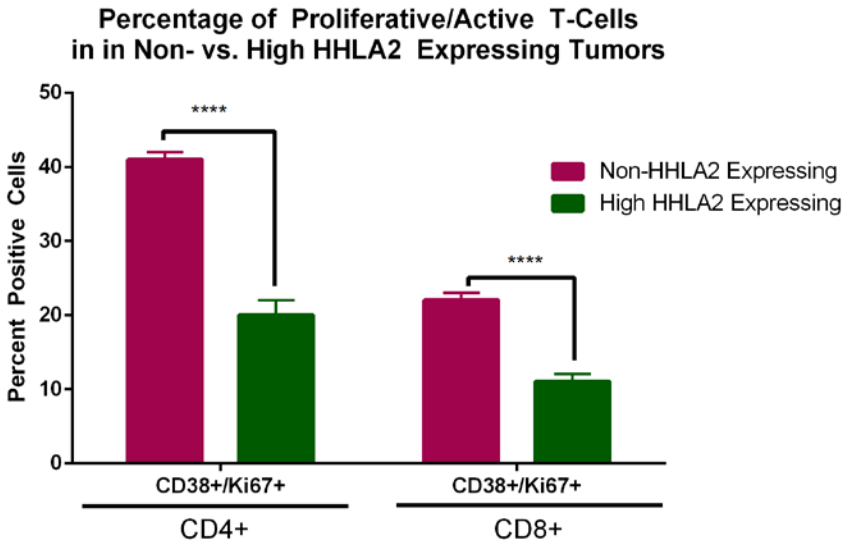
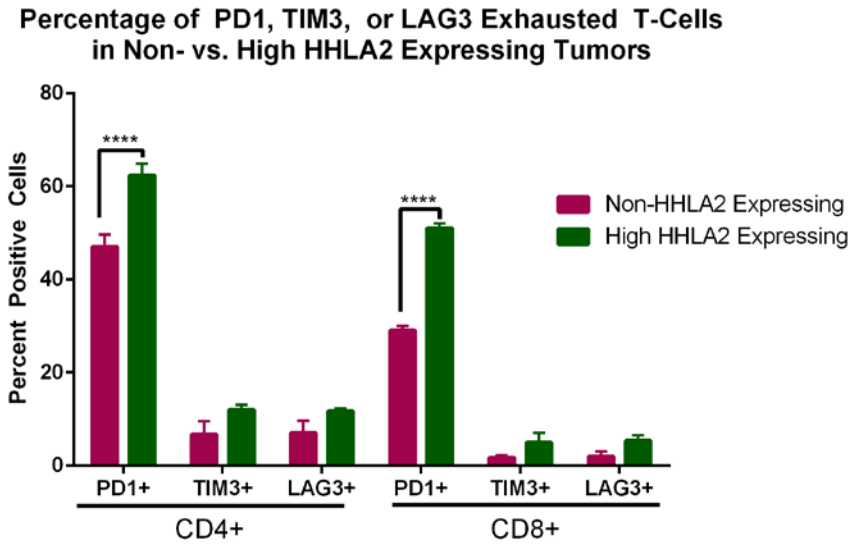
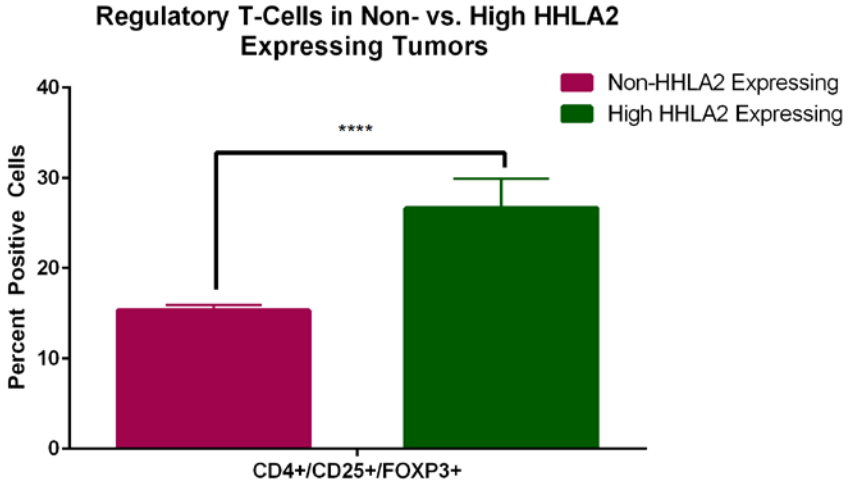
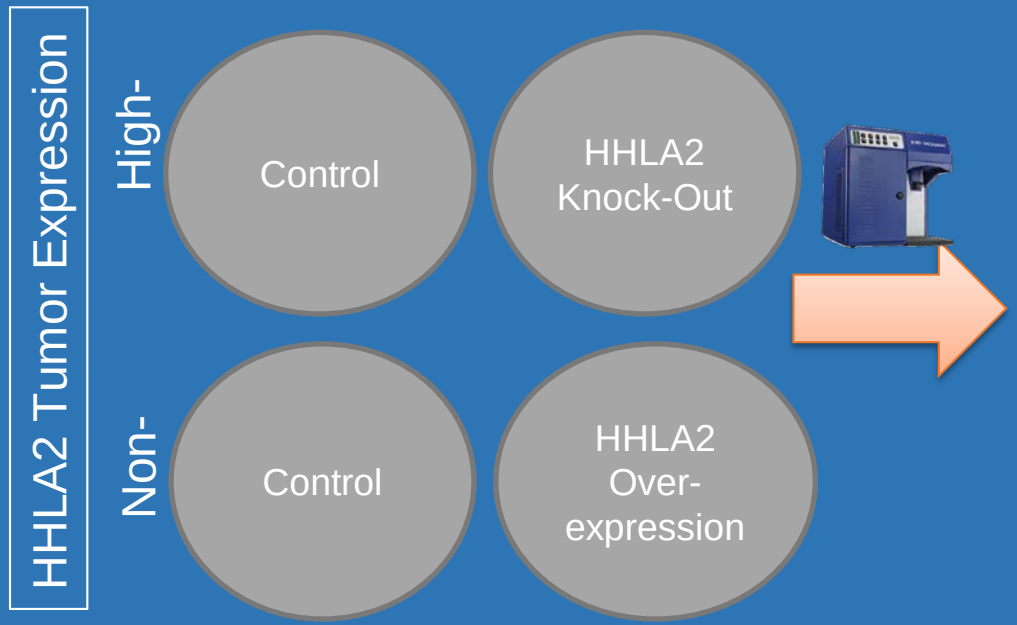






Aim 1.2: Examine the changes of T-cell sub-populations when HHLA2 is blocked within the TME.

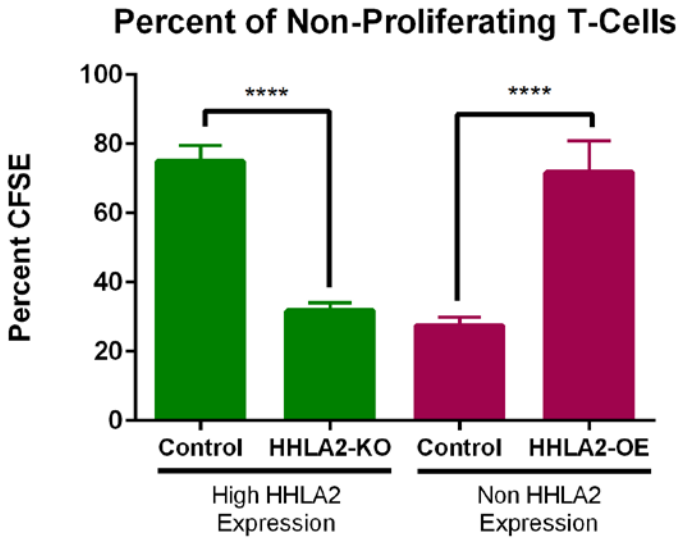
- Model: Human NSCLC Cells and Matched T-Cells
- Method: Genetically Modify NSCLC cell lines

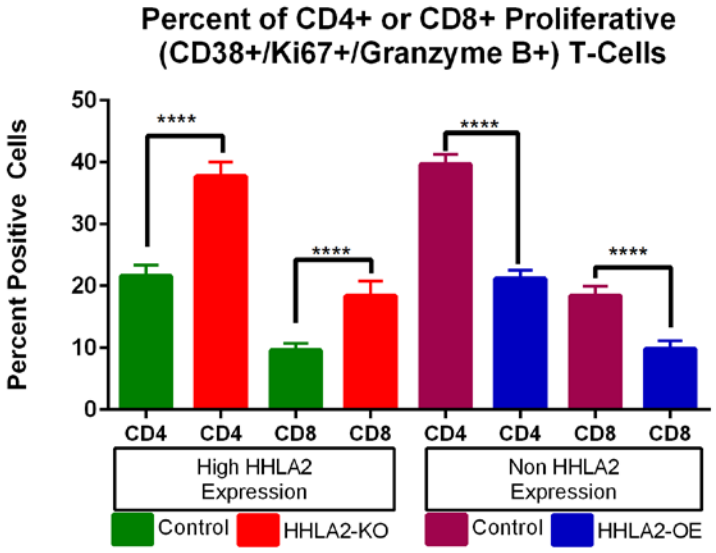


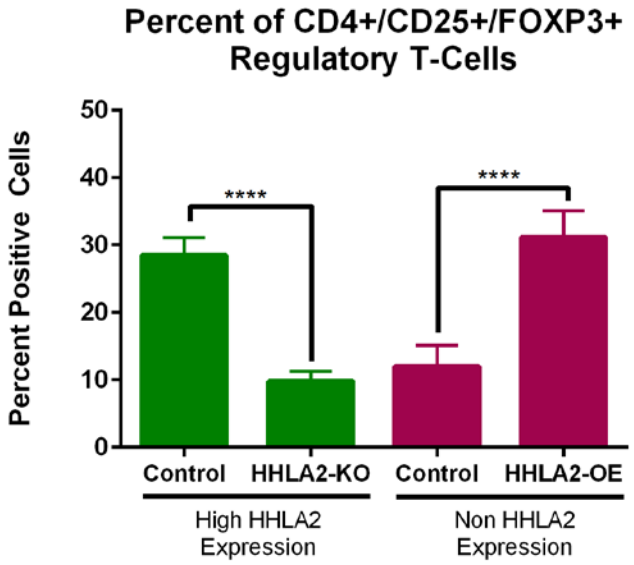
Analysis of T-Cell Subpopulations:

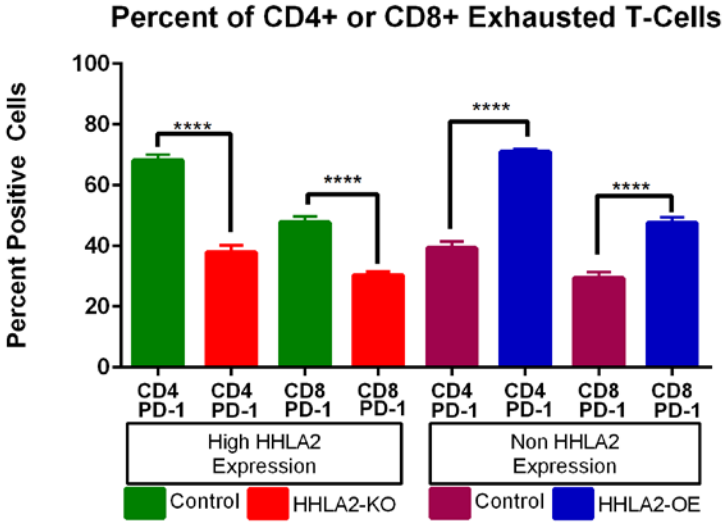
Flow Cytometry: CFSE and Markers of Ki67,Granzyme B, CD38, PD-1, TIM3, LAG-3, CTLA-4, CD25, and FoxP3

Aim 1.2: Expected Results





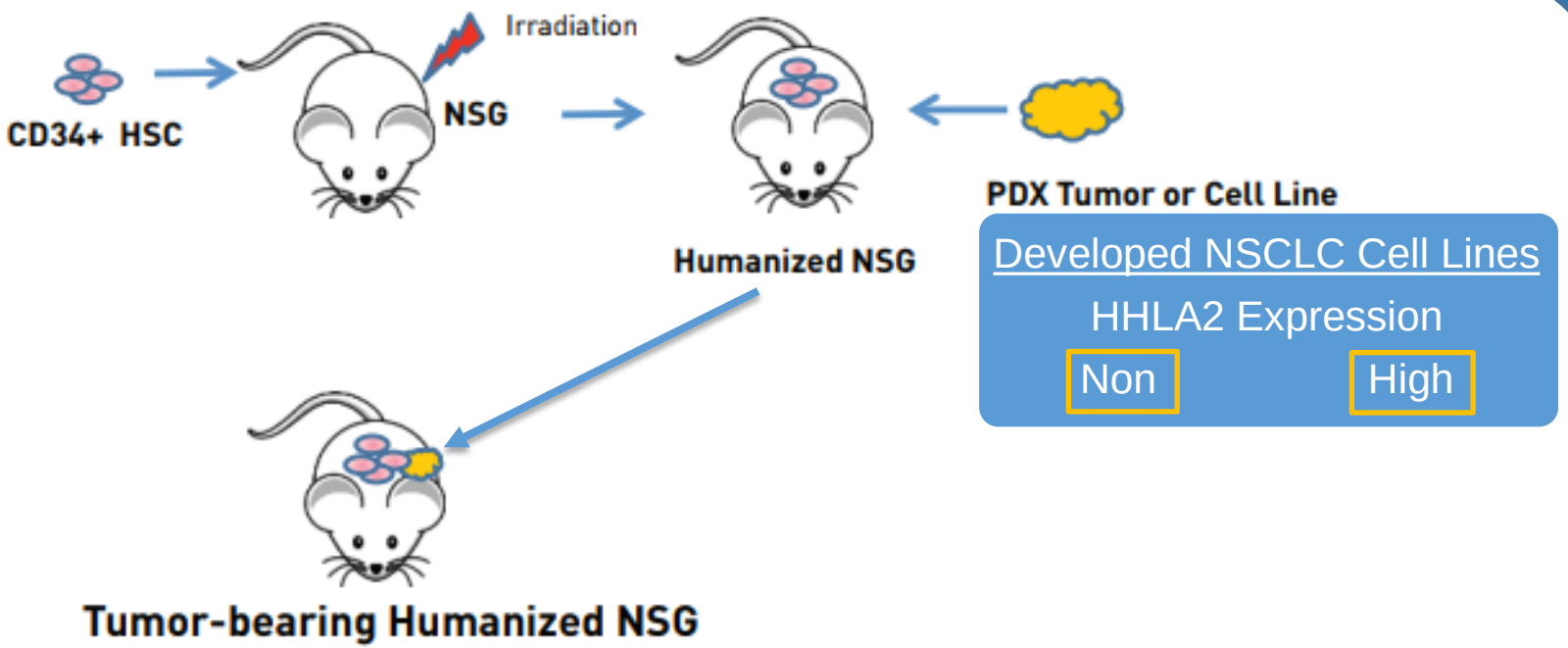




Aim 1.3: Investigate the activation of tumor infiltrating lymphocytes (TILs) or splenocytes of NSCLC bearing mice.

In Vivo Model

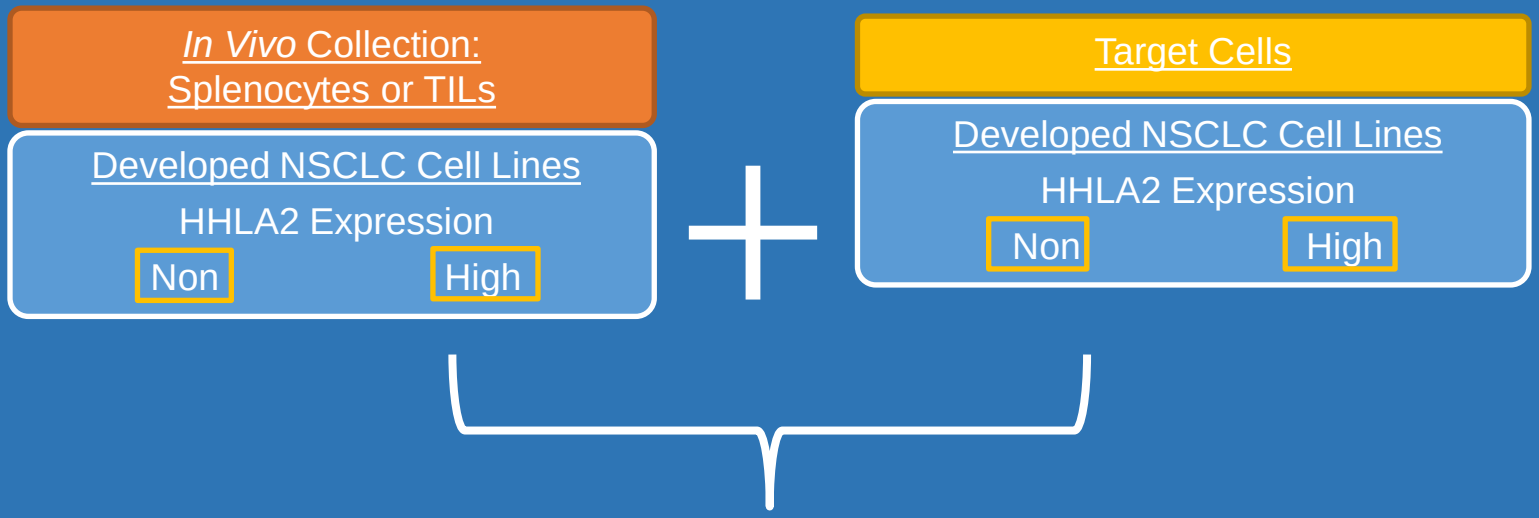
- HHLA2 is expressed in humans, but not in mice.
- Humanized NSG mice



Modified from the Jackson Laboratory

Aim 1.3: Investigate the activation of tumor infiltrating lymphocytes (TILs) or splenocytes of NSCLC bearing mice.

- Model: Humanized NSCLC Mouse Model
- Method: Co-Culture Experiments

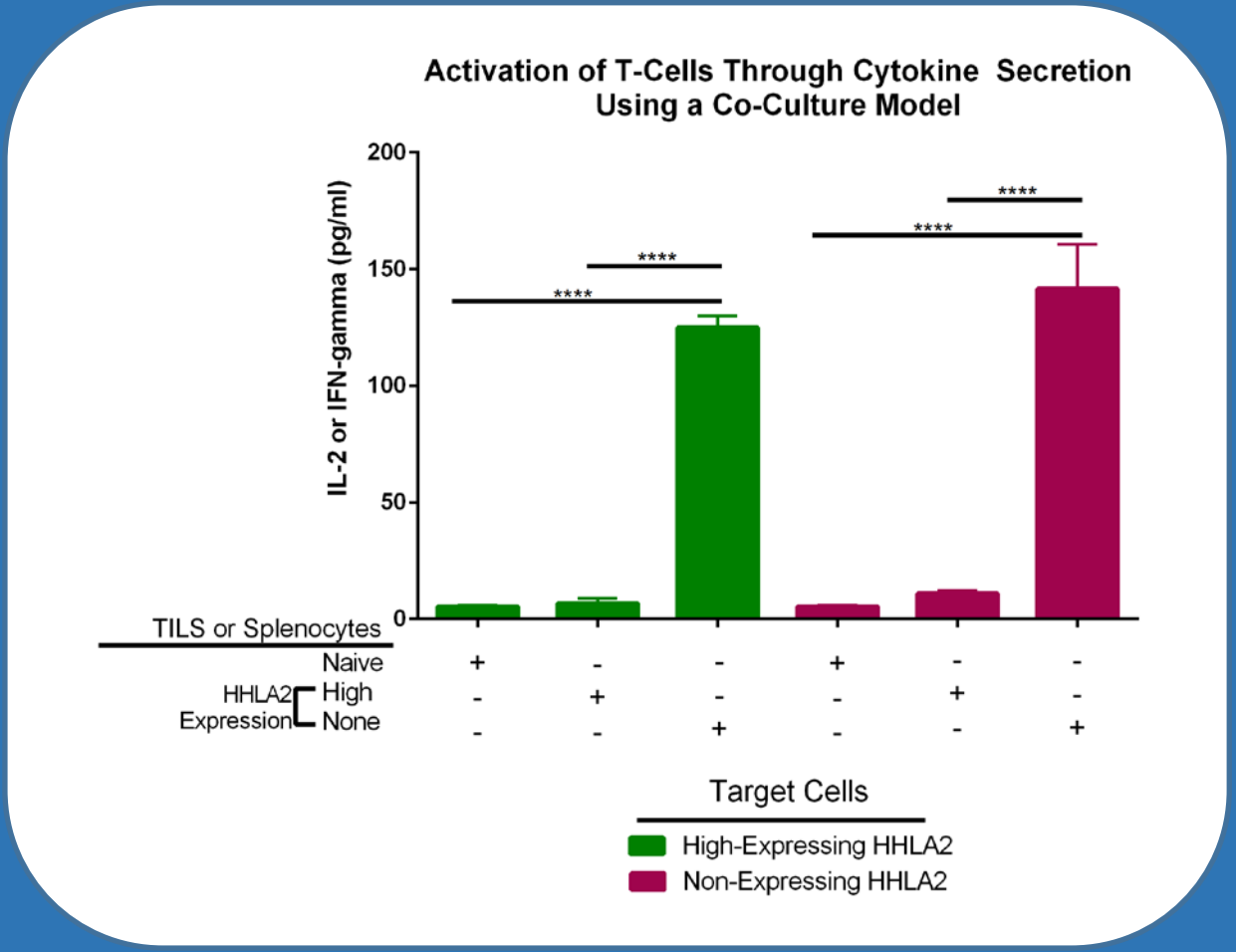


ELISA

1.IFNγ 2. IL-2

Aim 1.3: Expected Results

- The highest levels of IFNγ and IL-2 will be in the cells co-cultured with TILs or splenocytes from the mice implanted with the non-HHLA2 expressing cells.



Aim 1: Potential Pitfalls and Alternatives

- T-cell inactivation might be employed by PD-L1/PD-1 or CD80/CD86/CTLA-4 signaling, and cause HHLA2 inactivation of T-cells to be unrecognized.
  - PD-L1, PD-1, and CTLA-4 expression and function will be sought out as necessary.
  - Genetic or therapeutic blockage of PD-L1, PD-1, or CTLA-4 will be used.
- HHLA2 might be a silenced immune evasion mechanism.
  - Resistance models will be sought out.

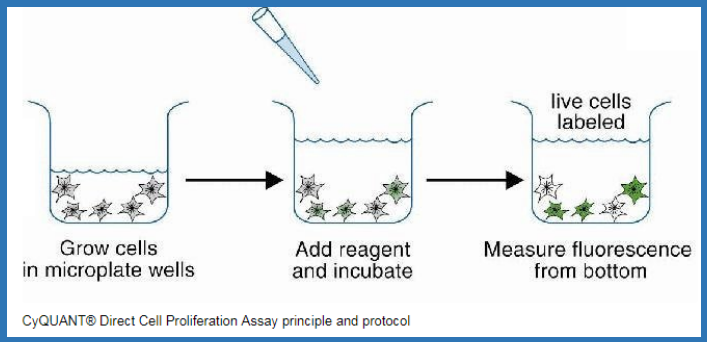
Aim 2.1: Test the therapeutic effect of a blocking HHLA2 in NSCLC.

- Model: Human NSCLC Cells and Matched T-Cells
- Methods:

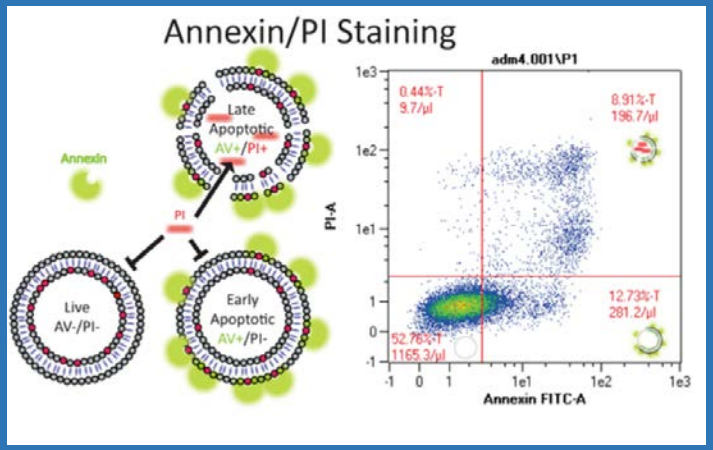
HHLA2 Tumor Expression

High-	Anti-CD3 + Control Ig	Anti-CD3 + anti-HHLA2 Ig
Intermediate-	Anti-CD3 + Control Ig	Anti-CD3 + anti-HHLA2 Ig
Non-	Anti-CD3 + Control Ig	Anti-CD3 + anti-HHLA2 Ig

Measure Cell Growth: CyQUANT Assay



Apoptosis: Annexin Assay



In Vivo Model

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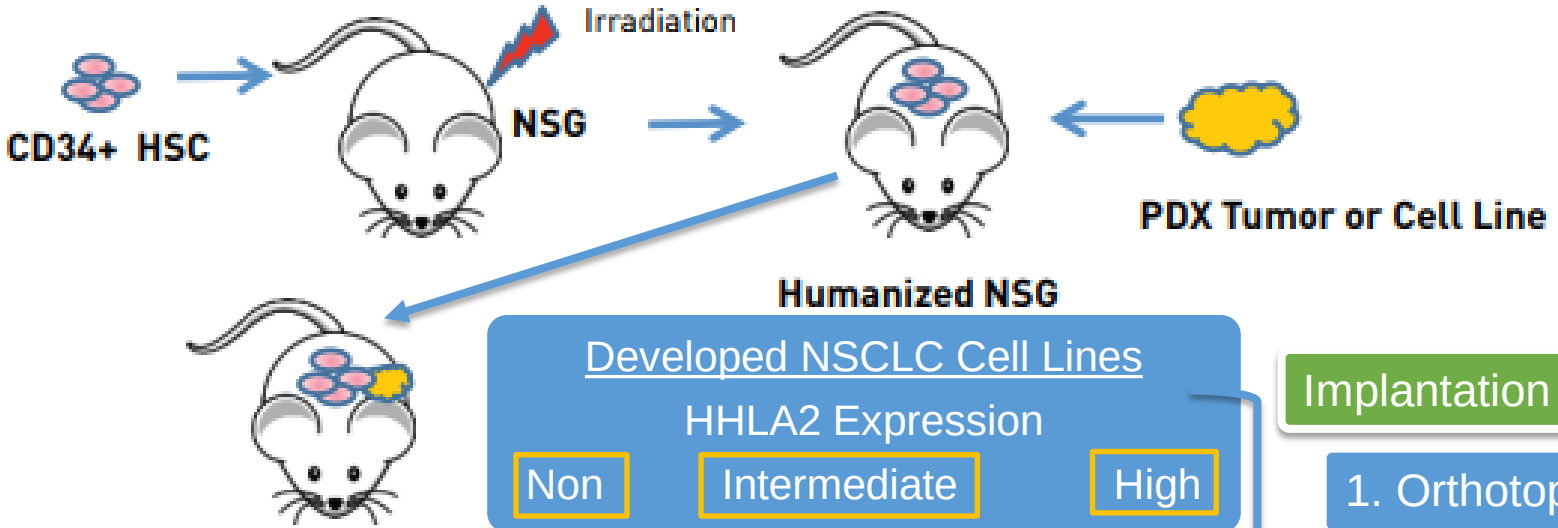
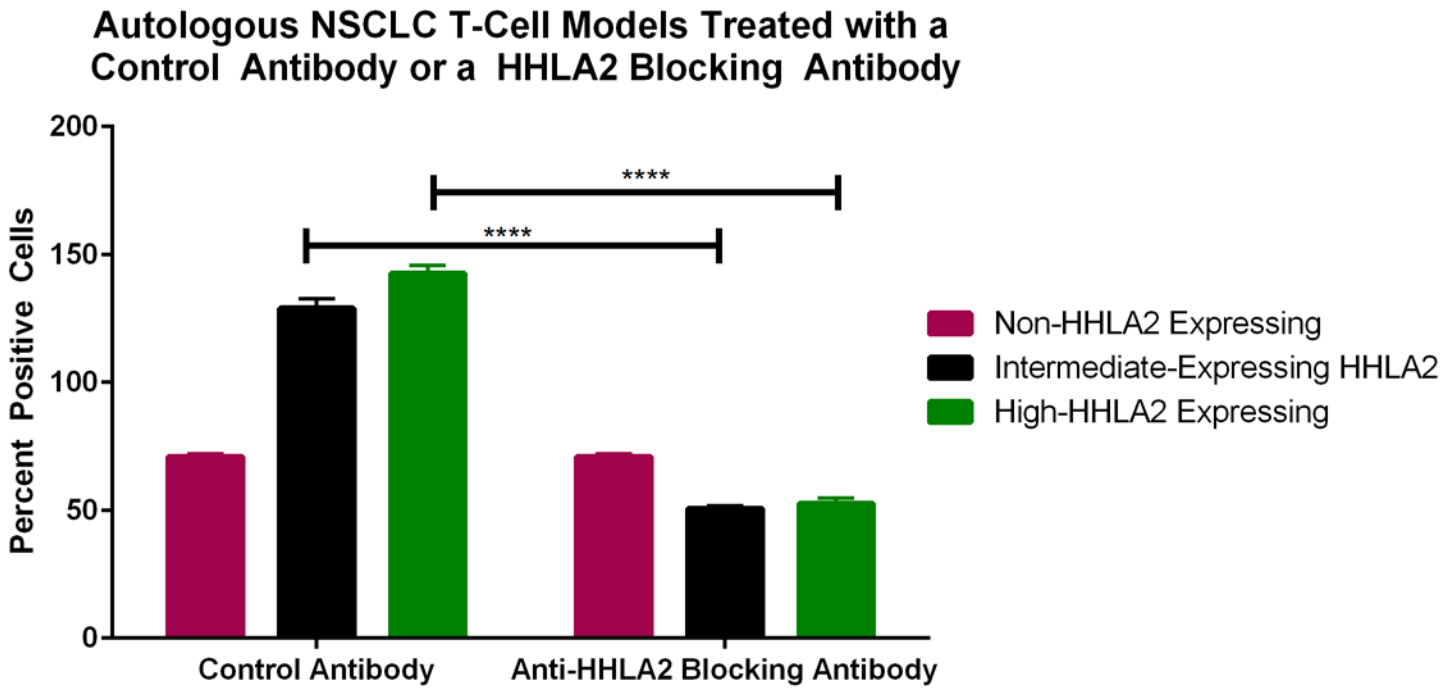
- Humanized NSG mice



Modified from the Jackson Laboratory

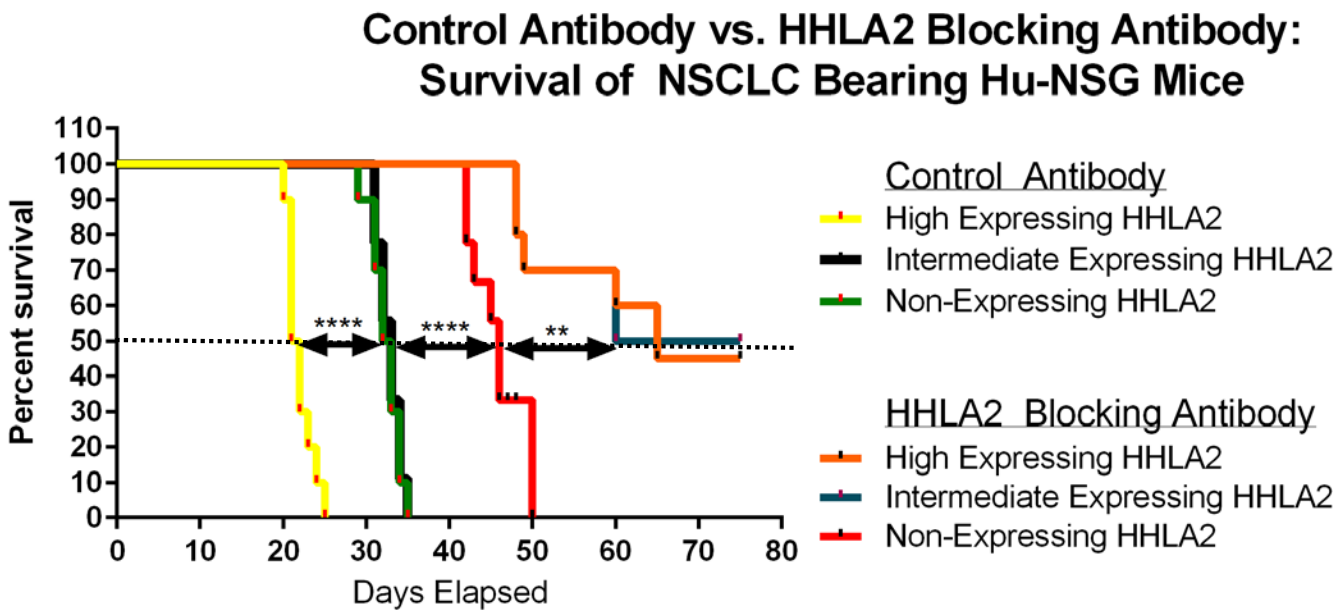
Aim 2.1: Expected Results

- I hypothesize that the HHLA high expressing cancer cells cultured with the HHLA2 blocking antibody will undergo less proliferation and more cell death compared to the control Ig group.



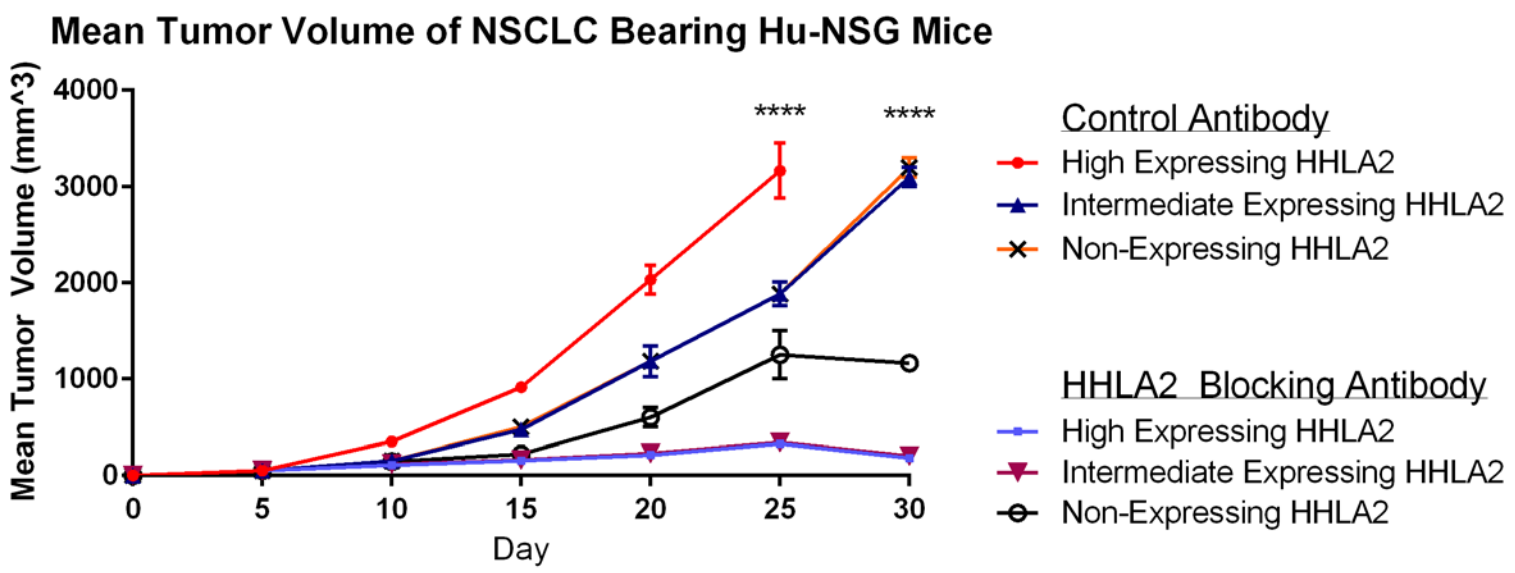
Aim 2.1: Expected Results

- I hypothesize that the HHLA2 blocking antibody will extend survival and yield long-term survivors.



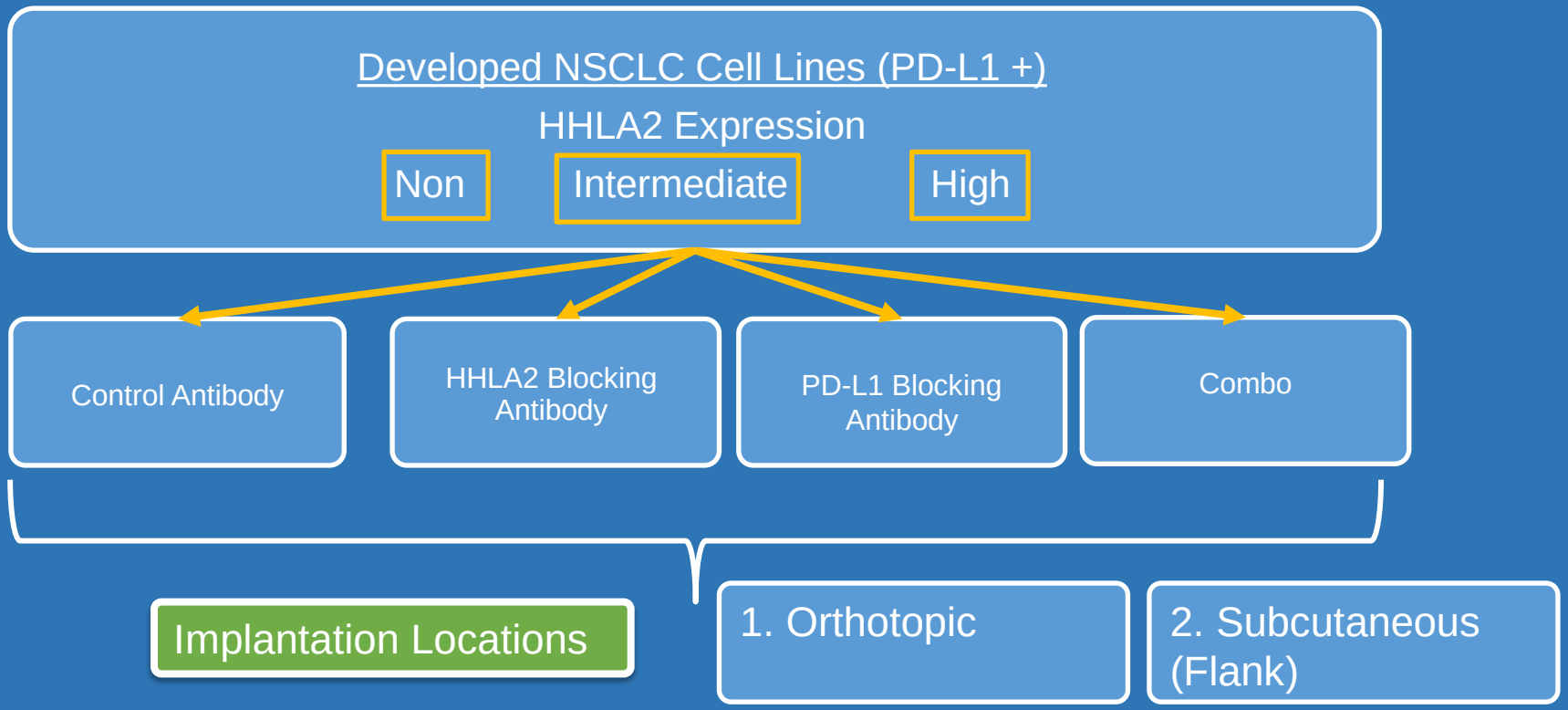
Aim 2.1: Expected Results

- I hypothesize that the HHLA2 blocking antibody will slow down tumor growth.



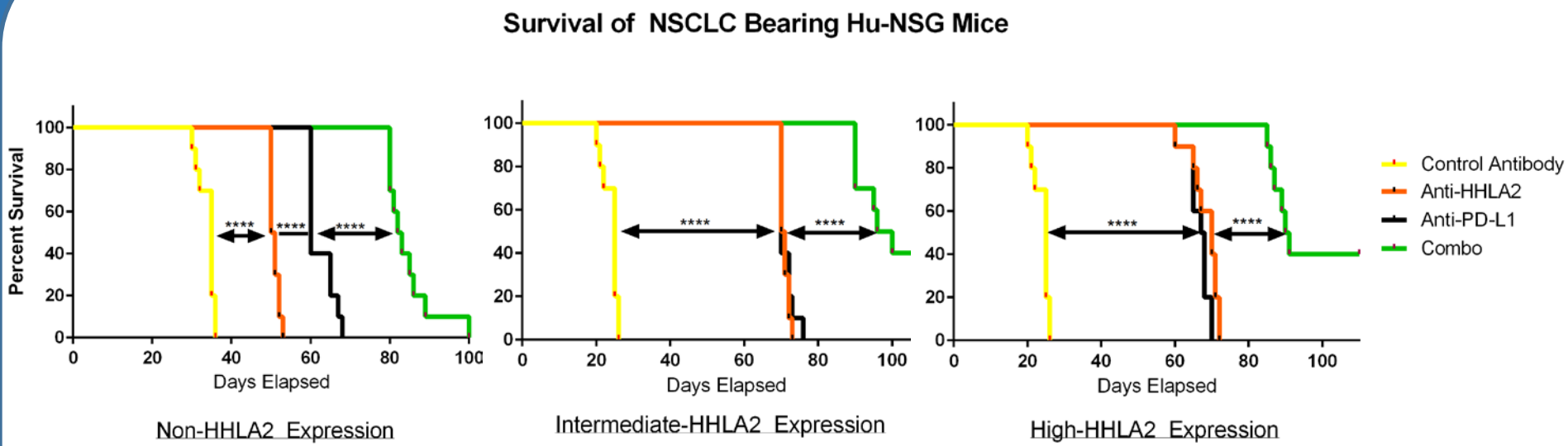
Aim 2.2: Investigate the therapeutic effect of targeting HHLA2 and PD-L1 *in vivo*.

- Model: Humanized NSG Mouse Model
- Methods: Survival Experiments and Tumor Measurements



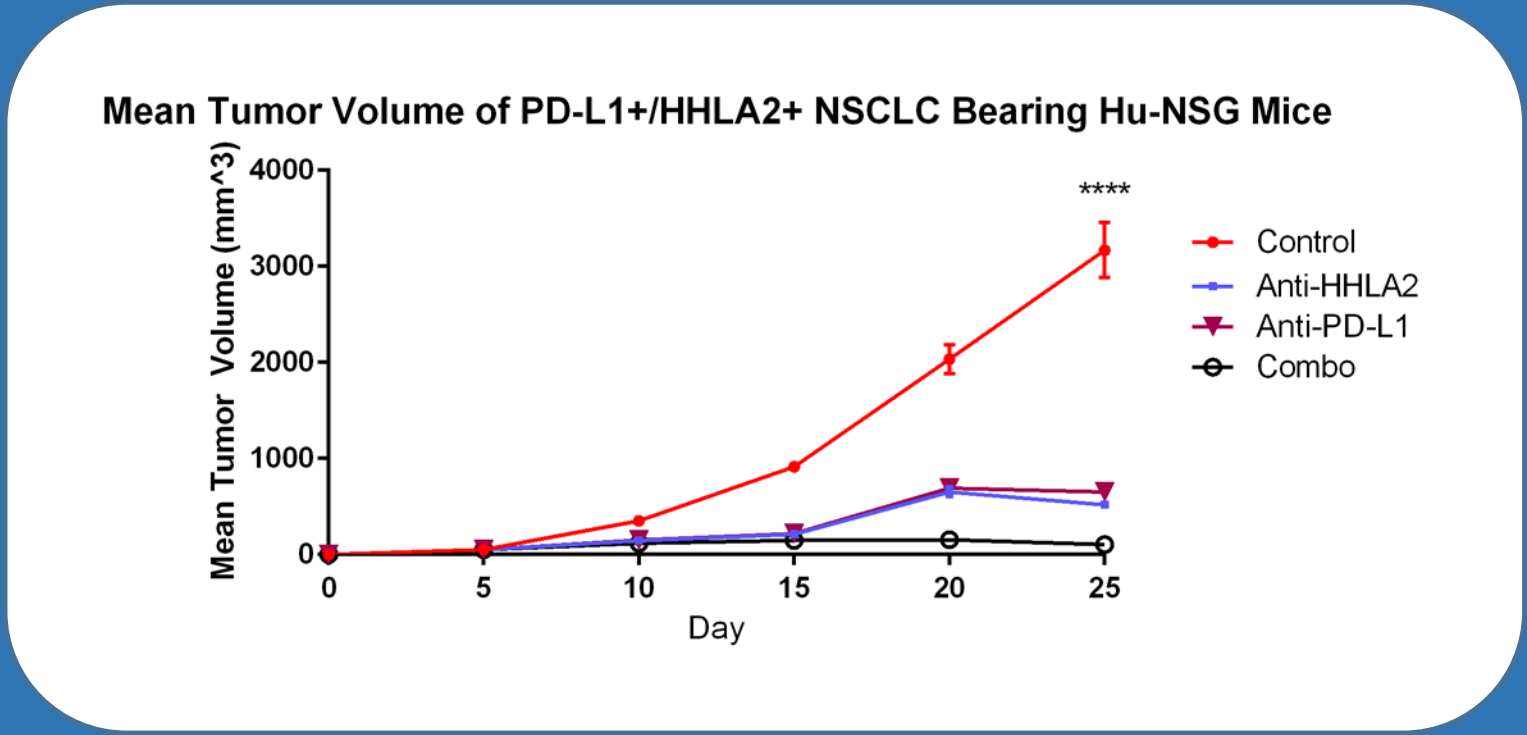
Aim 2.2: Expected Results

- I hypothesize that targeting both HHLA2 and PD-L1 in an HHLA2-overexpressing NSCLC model will extend the survival and yield long-term survivors compared to other treated groups.



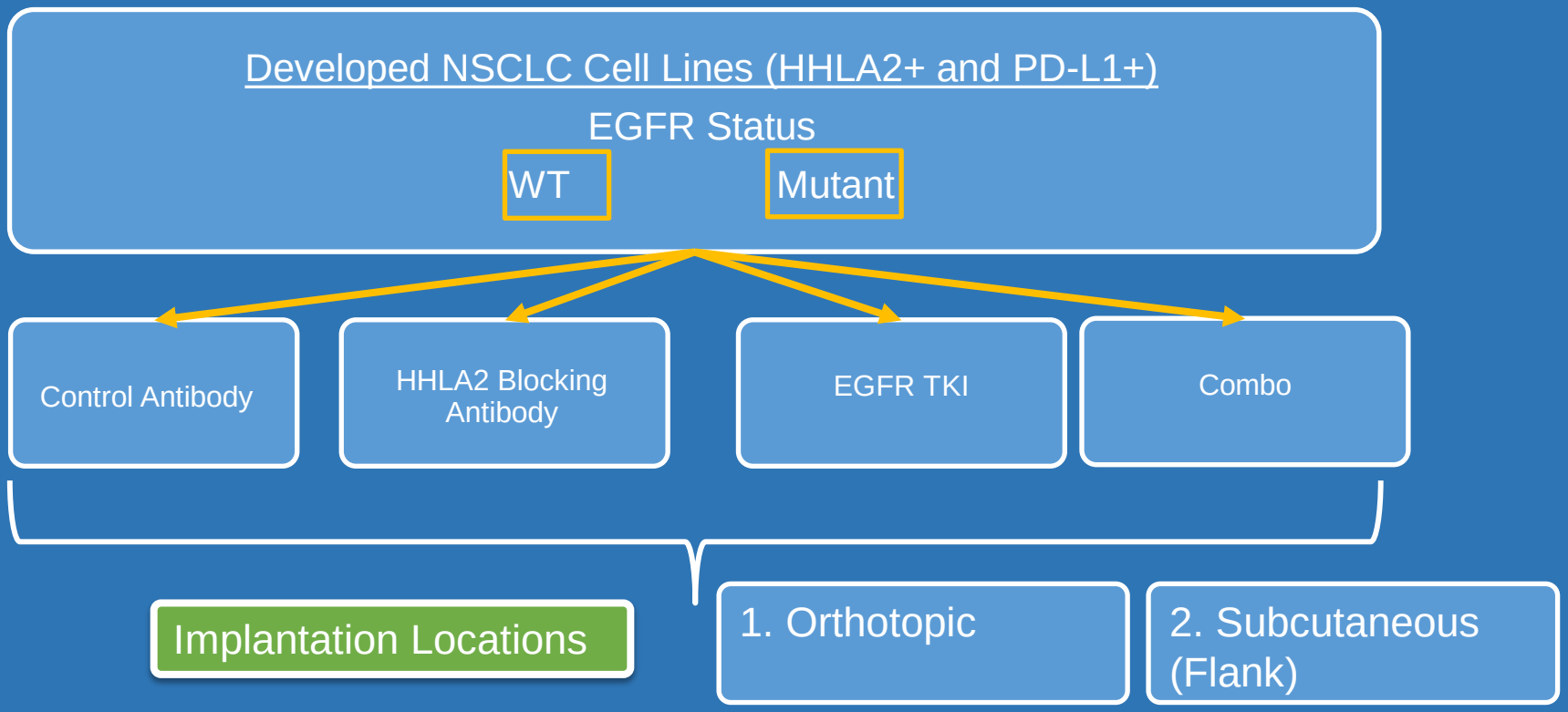
Aim 2.2: Expected Results

- I hypothesize that targeting both HHLA2 and PD-L1 in an HHLA2-overexpressing NSCLC model will delay growth of the tumor compared to other treated groups.



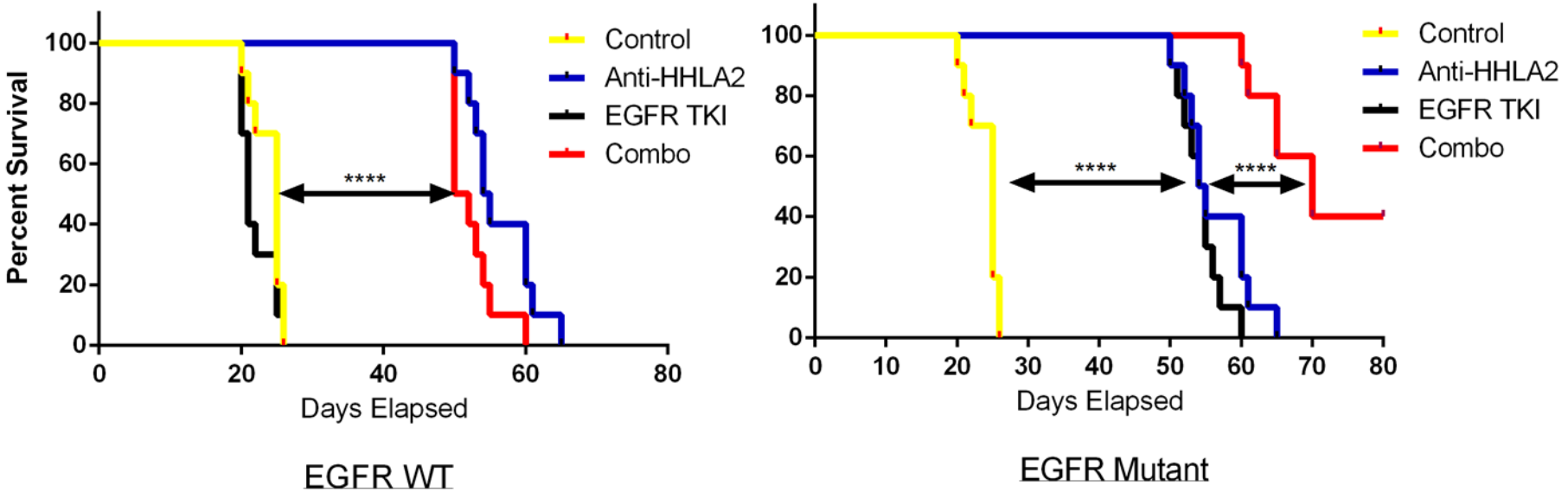
Aim 2.3: Inspect therapeutic effect of blocking EGFR and HHLA2 in EGFR mutant NSCLC *in vivo*.

- Model: Humanized Lung Cancer Mouse Model
- Methods: Survival Experiments and Tumor Measurements

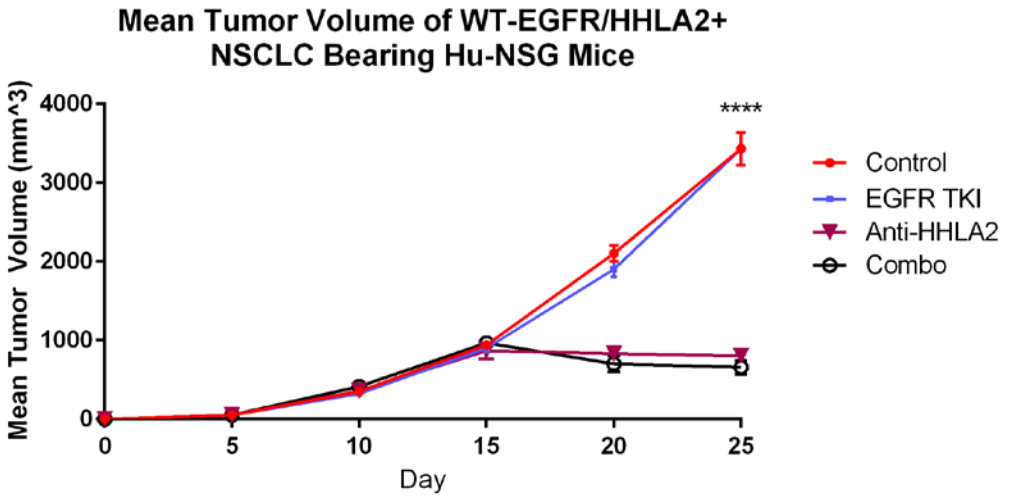


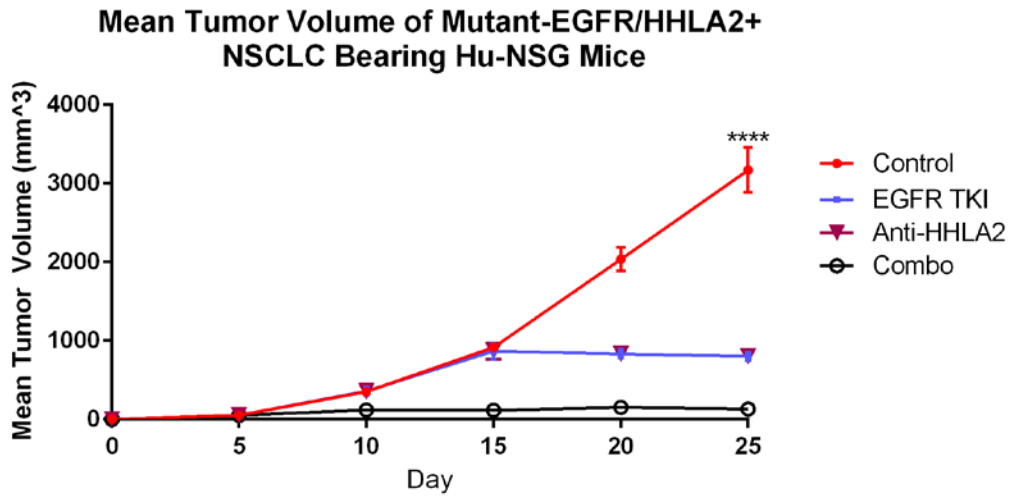
Aim 2.3: Expected Results

- I hypothesize that treatment using an EGFR TKI and an HHLA2 blocking antibody in a EGFR mutant NSCLC model will extend the survival of the mice compared to the other treatment groups based on the theory that HHLA2 is highly expressed on EGFR mutant cells for tumor evasion.



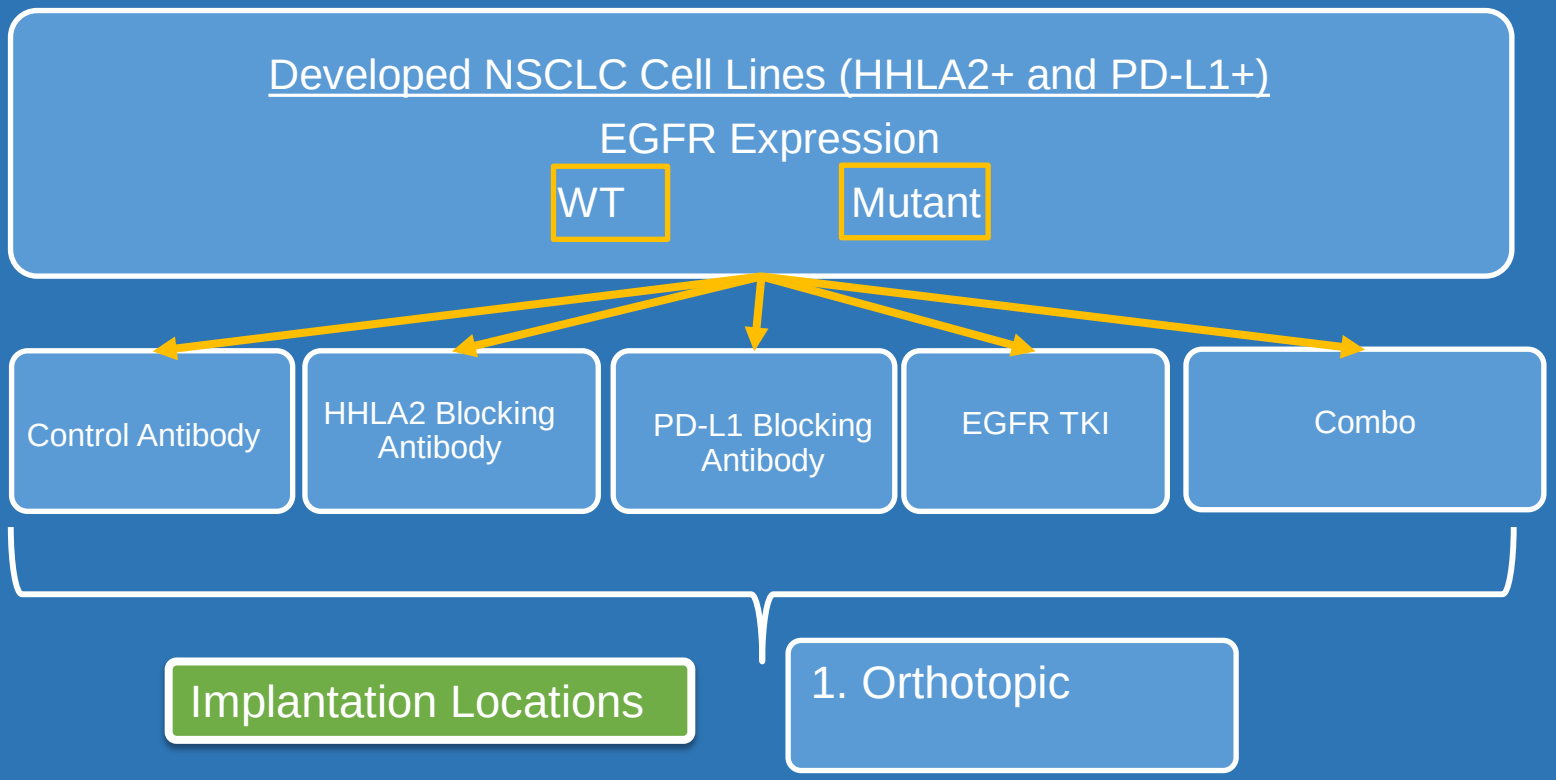
Aim 2.3: Expected Results





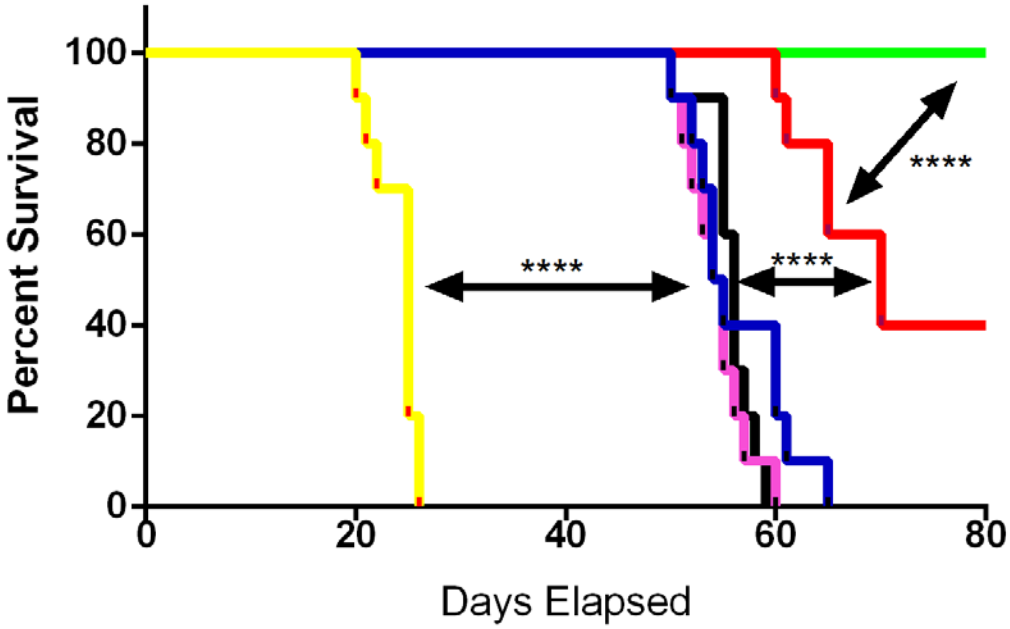
Aim 2.3 Extension: Inspect therapeutic effect of blocking EGFR, PD-L1, and HHLA2 in EGFR mutant NSCLC *in vivo*.

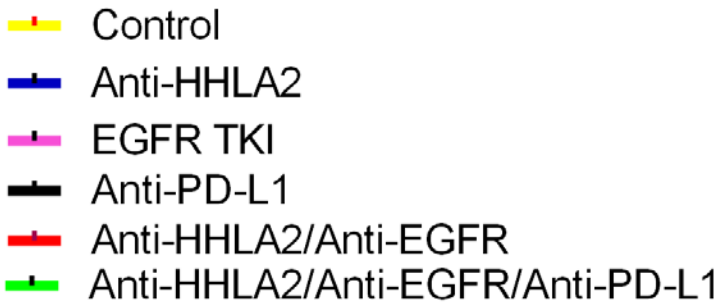
- Model: Orthotopic Lung Cancer Mouse Model
- Methods: Survival Experiments and Tumor Measurements



Aim 2.3 Extension: Expected Results

Survival of EGFR Mutant/PD-L1+/HHLA2+ NSCLC Bearing Hu-NSG Mice





EGFR Mutant/PD-L1+/HHLA2+

Aim 2: Potential Pitfalls and Alternatives

- Genetic variability might be seen within the PDX models.
  - Use of the genetically modified models of Aim 1.2 will be utilized instead.
- HHLA2 activation might arise in response to anti-PD-L1 or anti-EGFR treatment.
  - Anti-PD-L1 or anti-EGFR resistant models will be developed.
  - Sequential treatment of anti-PD-L1 or anti-EGFR followed by anti-HHLA2 will be utilized.

Alternative Strategies

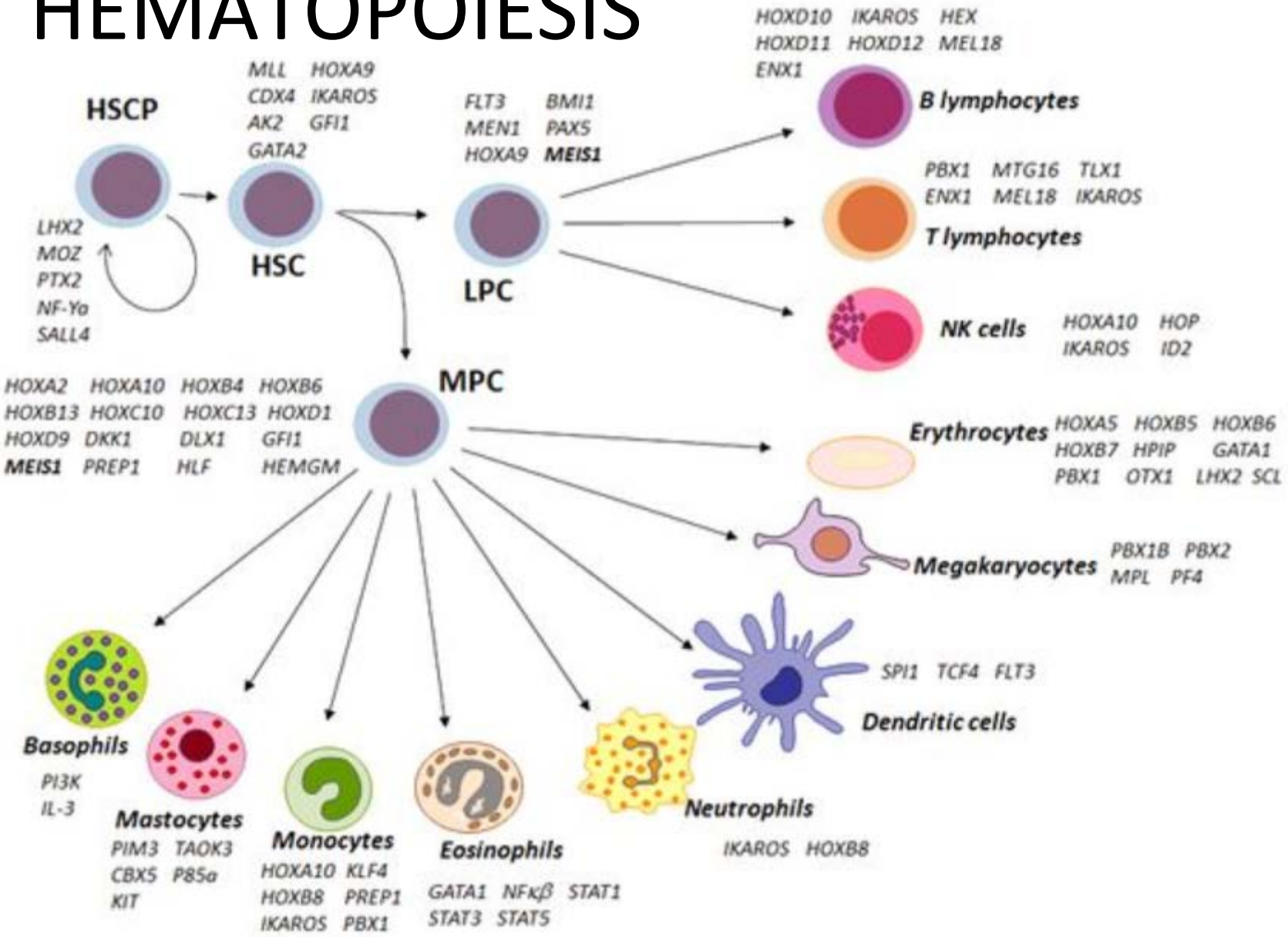
- Explore other aspects of the TME.
  - MDSCs, B-Cells, NK Cells
- Block other members of the B7 family, B7x or B7-H3, in NSCLC.

Conclusions

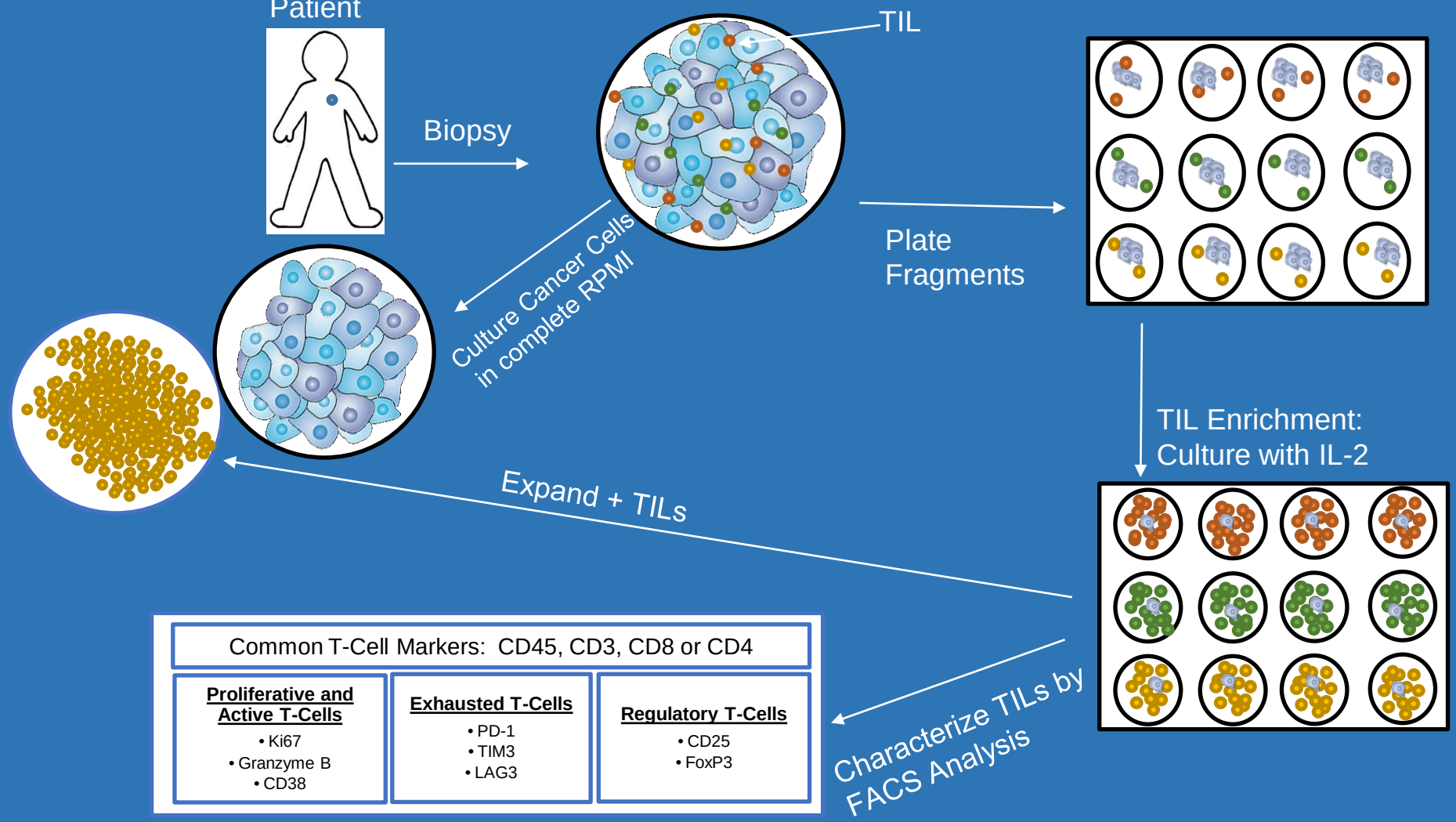
- HHLA2 is a recently discovered member of the B7 family of T-cell inhibitory molecules.
- HHLA2 is widely expressed in NSCLC, and may provide a novel therapeutic immuno-target.
- Successful results from targeting HHLA2 in NSCLC immunocompetent mouse models may lead to clinical trials and exploration of HHLA2 in other cancers.

Thank You!

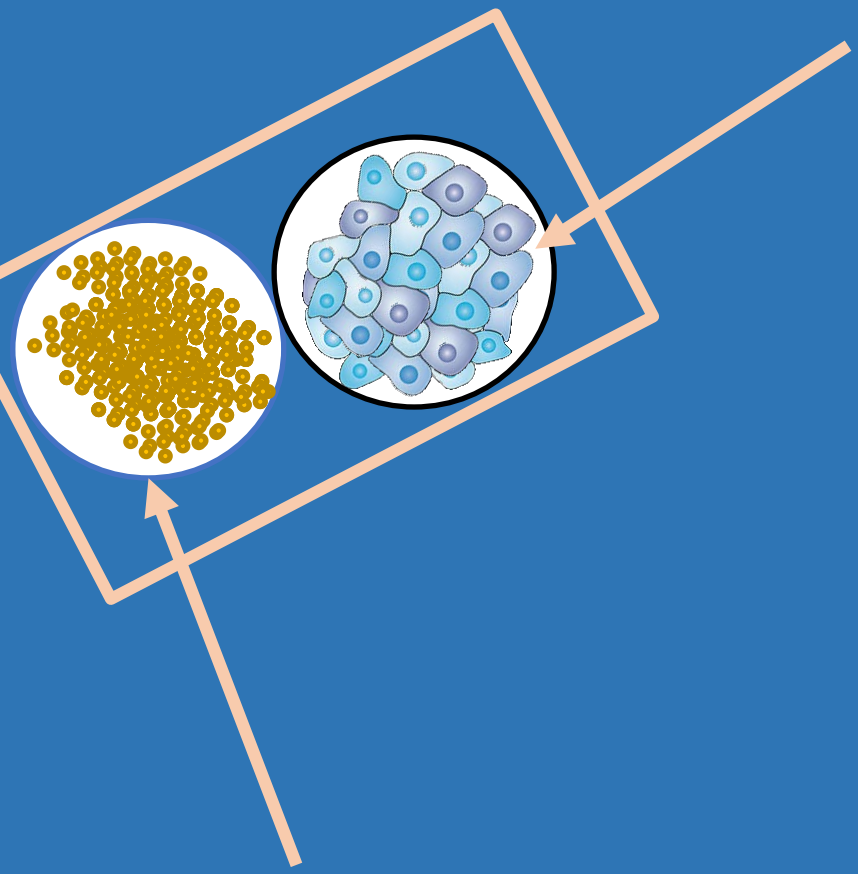
HEMATOPOIESIS



Development of lung cancer cell lines and matched T-cells



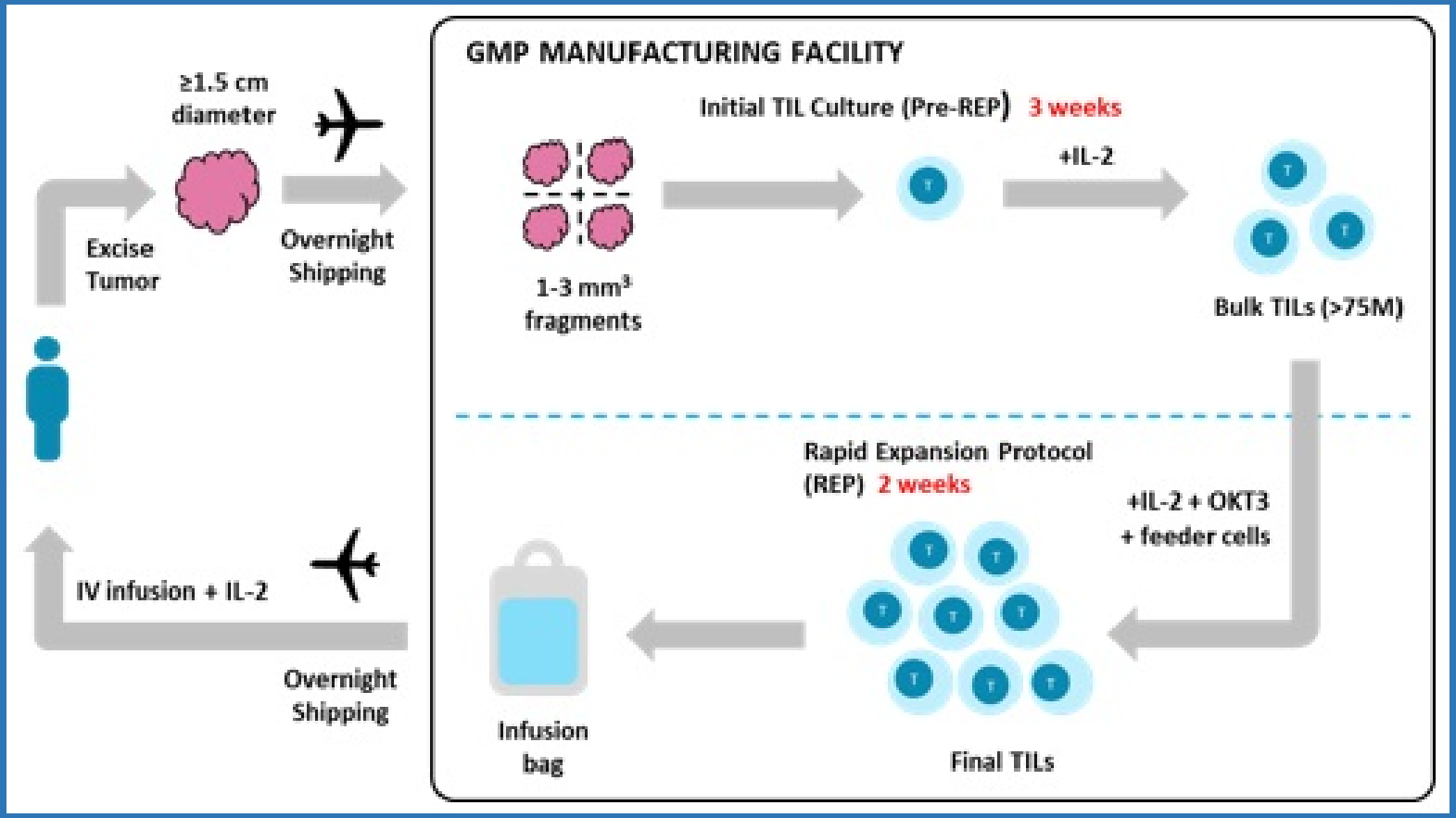
The Human Lung Cancer Model: Cancer Cells with Matched T-Cells



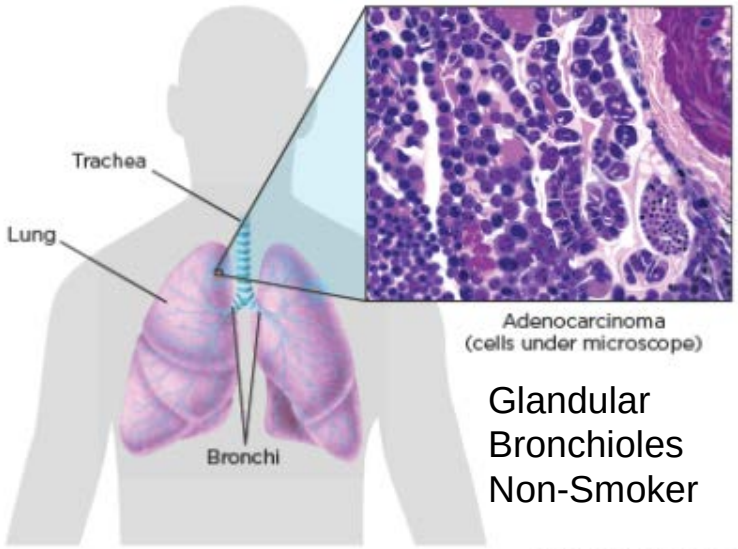
Lung Cancer Cells

Matched T-Cells

Rapid Expansion

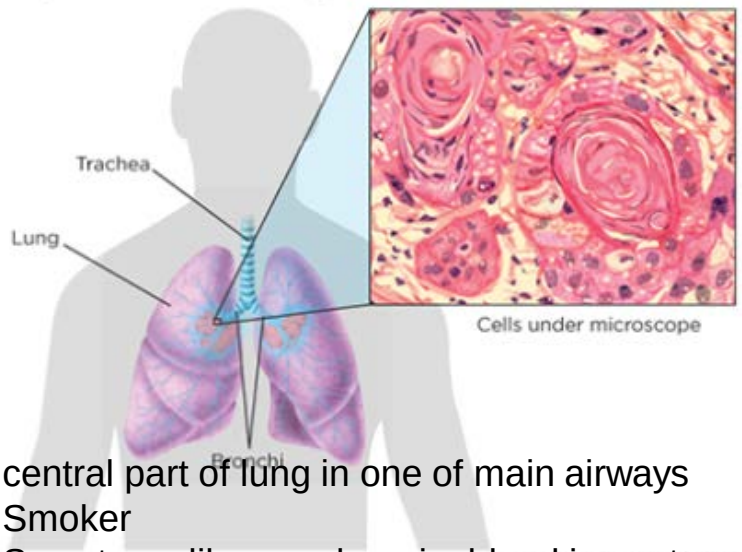


Adenocarcinoma



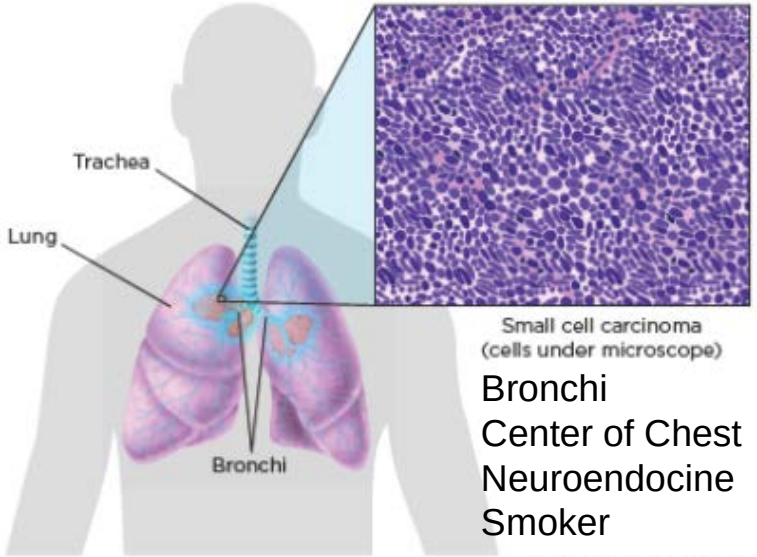
Glandular
Bronchioles
Non-Smoker

Squamous Cell Lung Cancer

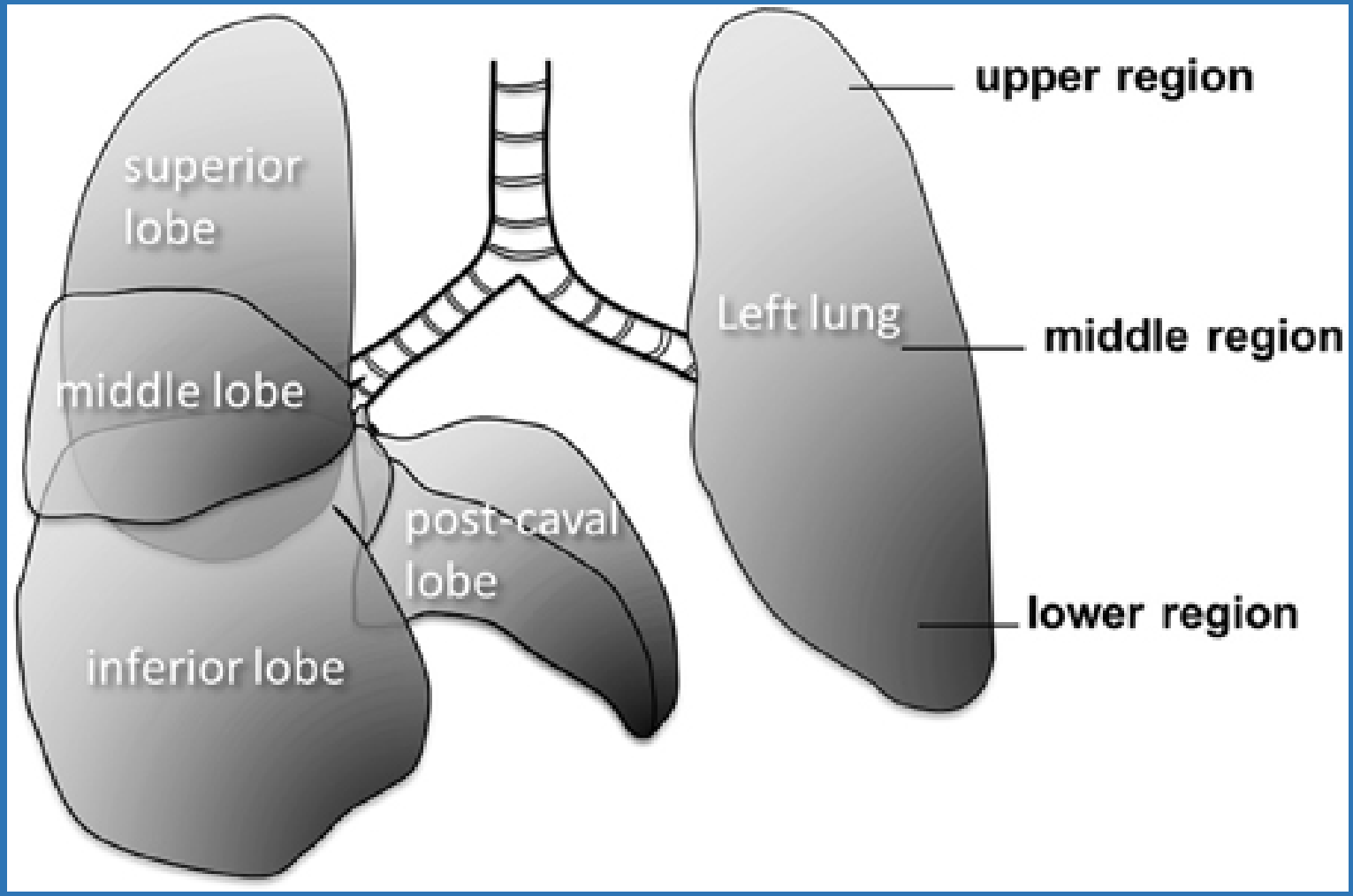


central part of lung in one of main airways
Smoker
Symptoms like cough, pain, blood in sputum,

Small Cell Carcinoma



Bronchi
Center of Chest
Neuroendocine
Smoker


superior lobe
middle lobe
inferior lobe
post-caval lobe
Left lung
upper region
middle region
lower region

Lung Cancer Treatment Algorithm

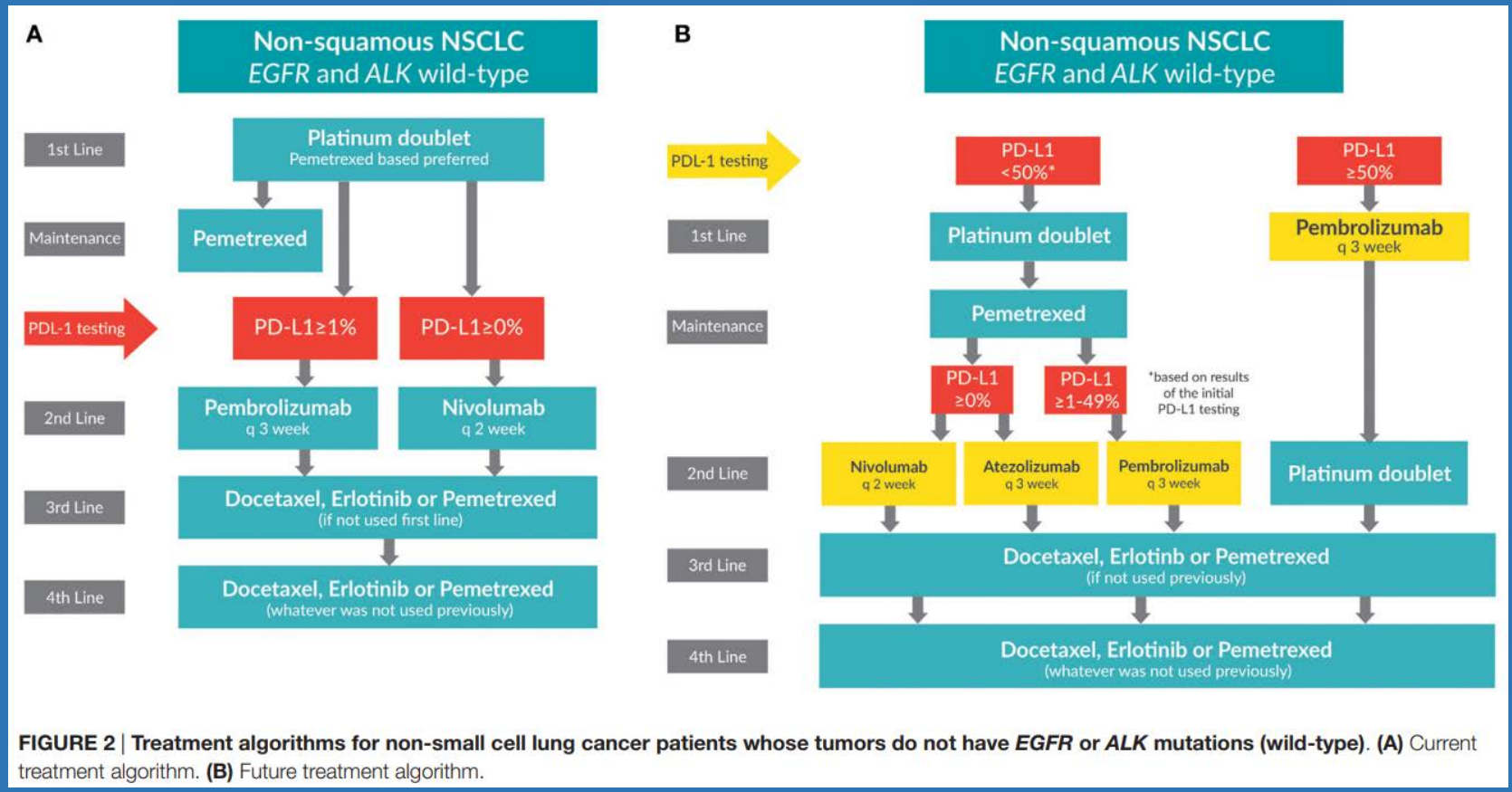
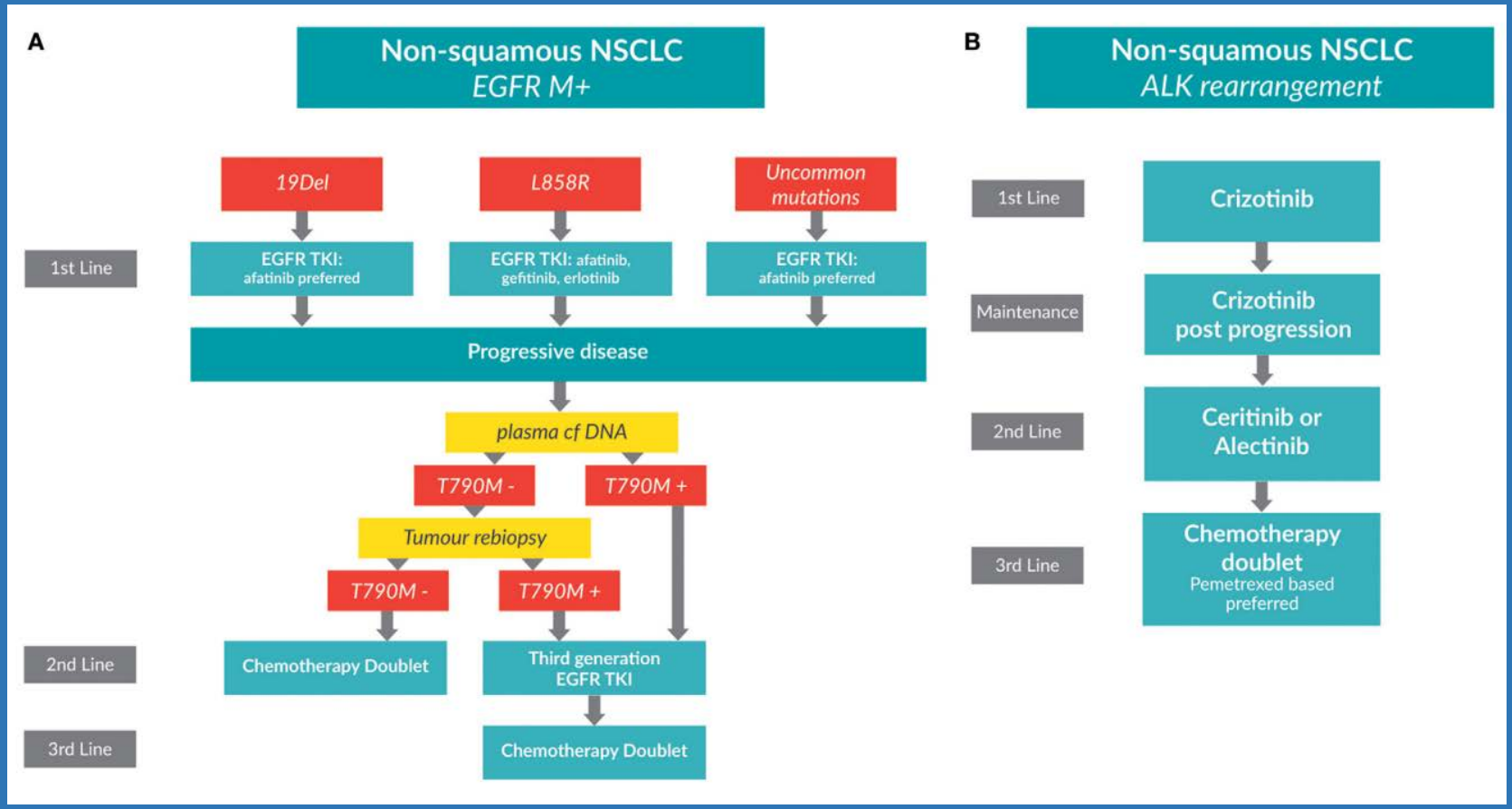


FIGURE 2 | Treatment algorithms for non-small cell lung cancer patients whose tumors do not have *EGFR* or *ALK* mutations (wild-type). (A) Current treatment algorithm. **(B)** Future treatment algorithm.

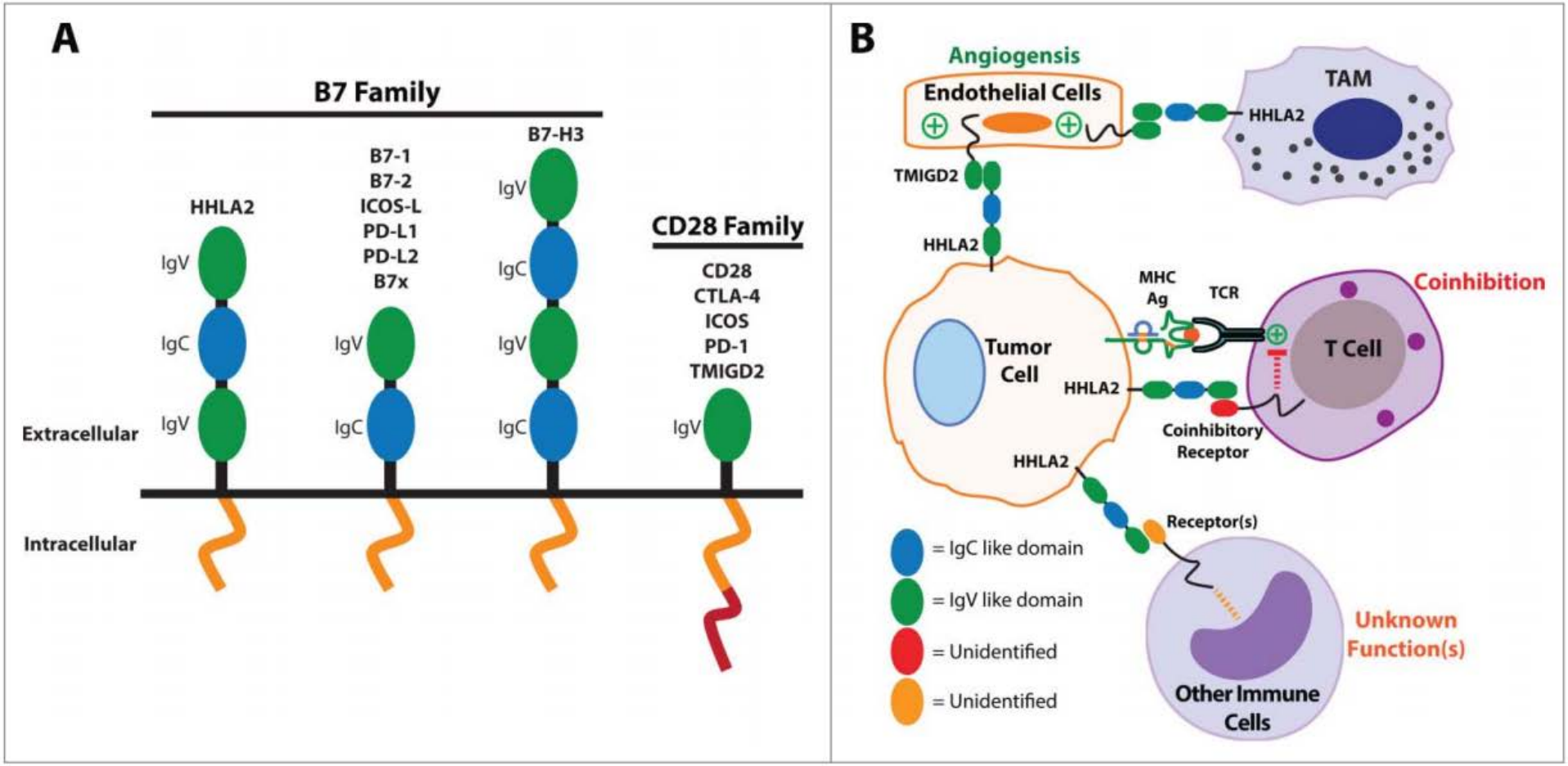
Melosky, Frontiers in Oncology, 2017

Lung Cancer Treatment Algorithm

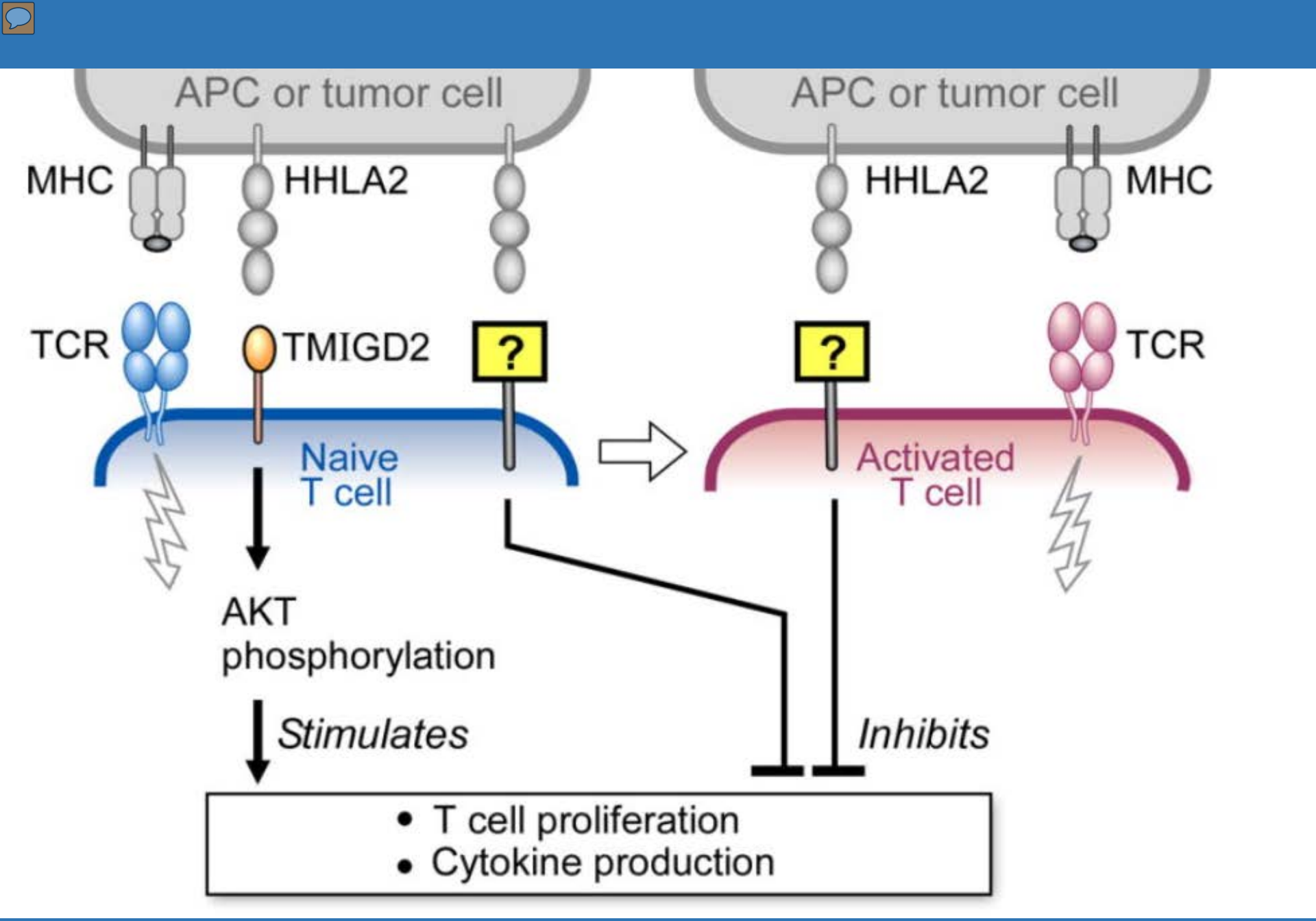


Melosky, Frontiers in Oncology, 2017

HHLA2 Signaling

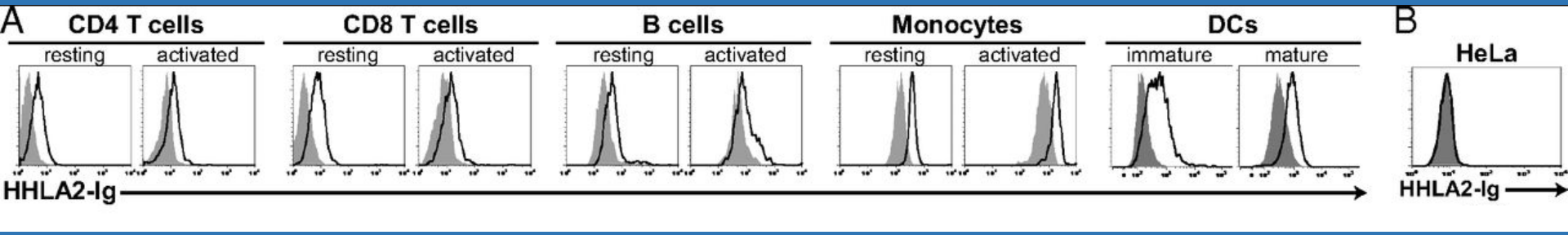


Janakiram, Oncoimmunology, 2015



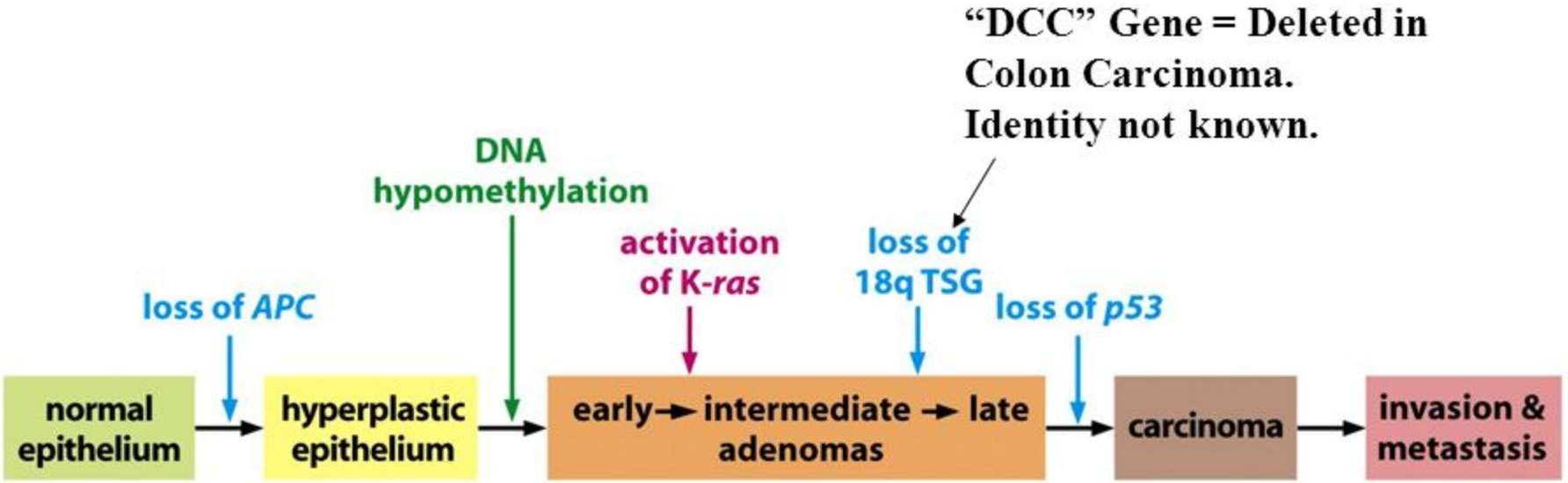
Xiao and Freeman, 2015, AACR

A Putative Receptor Exists for HHLA2



Loss of Tumor Suppressor Genes (TSG) in Progression in Colon Carcinoma

(See Also Sidebar 11.1, p. 434 Relating p53 loss to RAS mutations in the same cancer cell.)

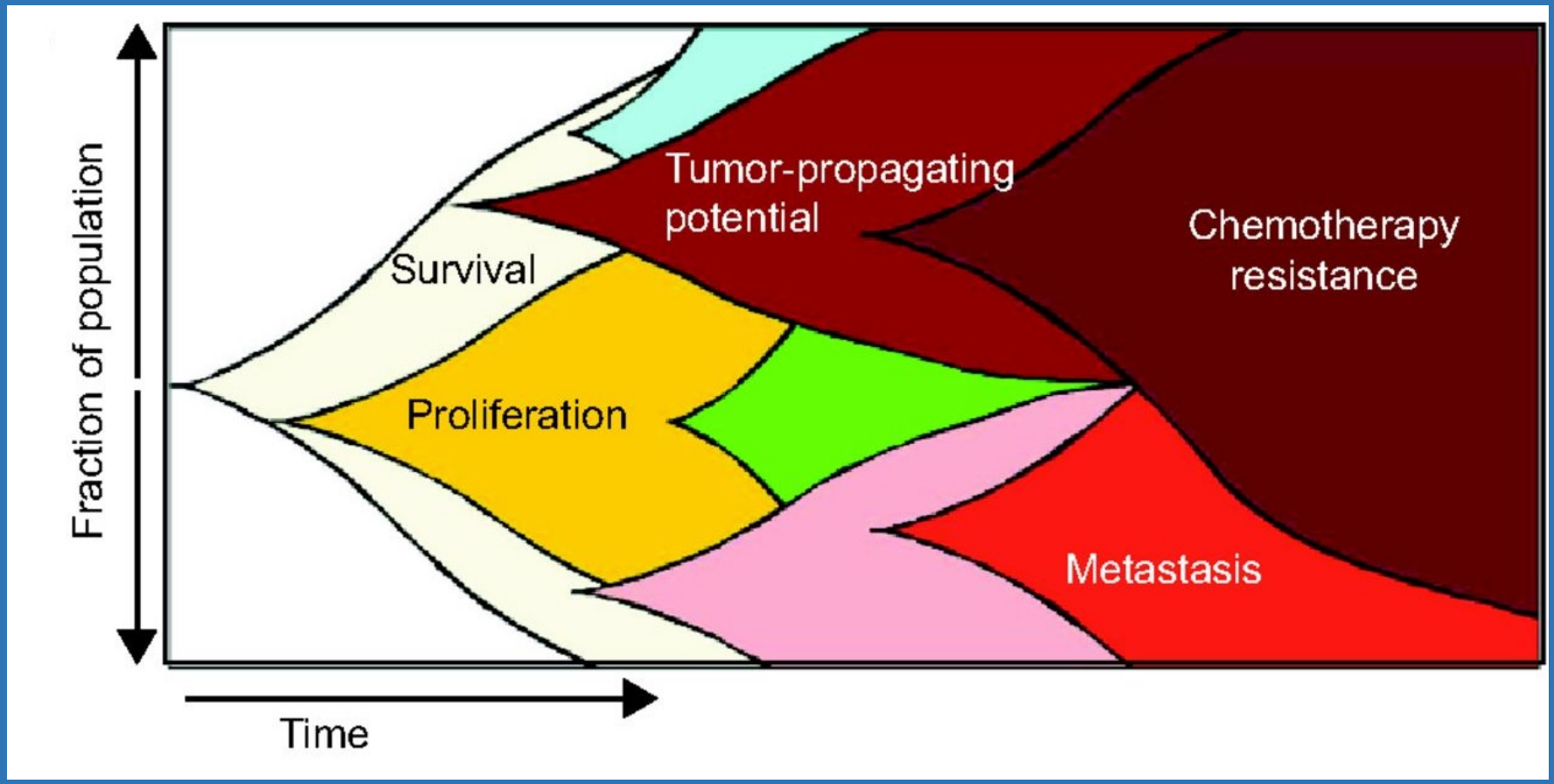


"APC" = Adenomatous polyposis coli gene (Cancer suppressor gene)
"K-ras" = Oncogene activated, transduced, or mutated, first identified in virally-induced rat sarcoma. (On chromosome 1*)
TSG = Tumor Suppressor Gene
p53 = Major cancer suppressor gene

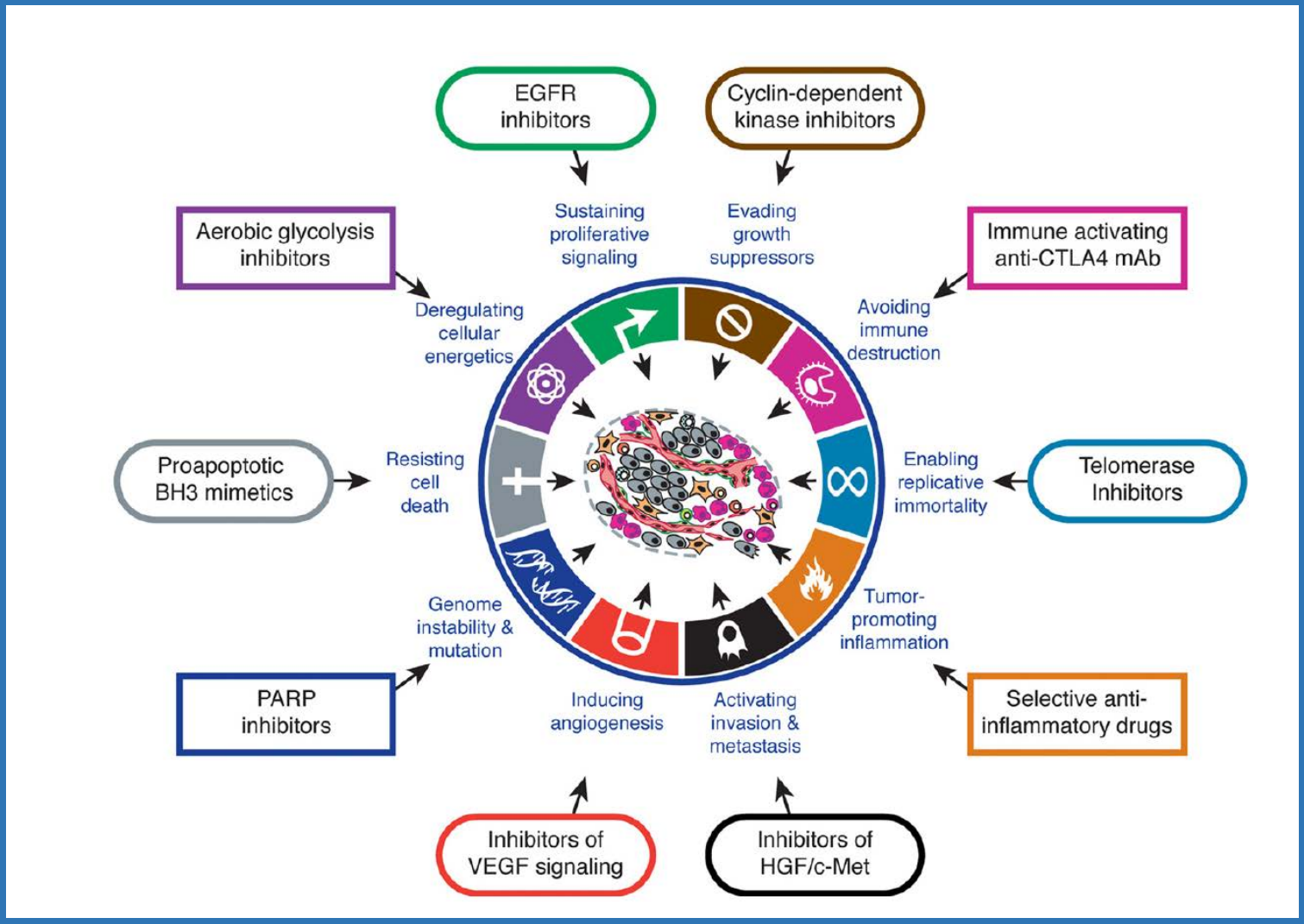
*EMBO J. 1983; 2(12): 2281–2283.
PMCID: PMC555446
Localisation of the human N-ras oncogene to chromosome 1cen - p21 by in situ hybridisation.
M Davis, S Malcolm, A Hall, and C J Marshall

Figure 11.10 *The Biology of Cancer* (© Garland Science 2007)

Clonal Expansion of Cancer



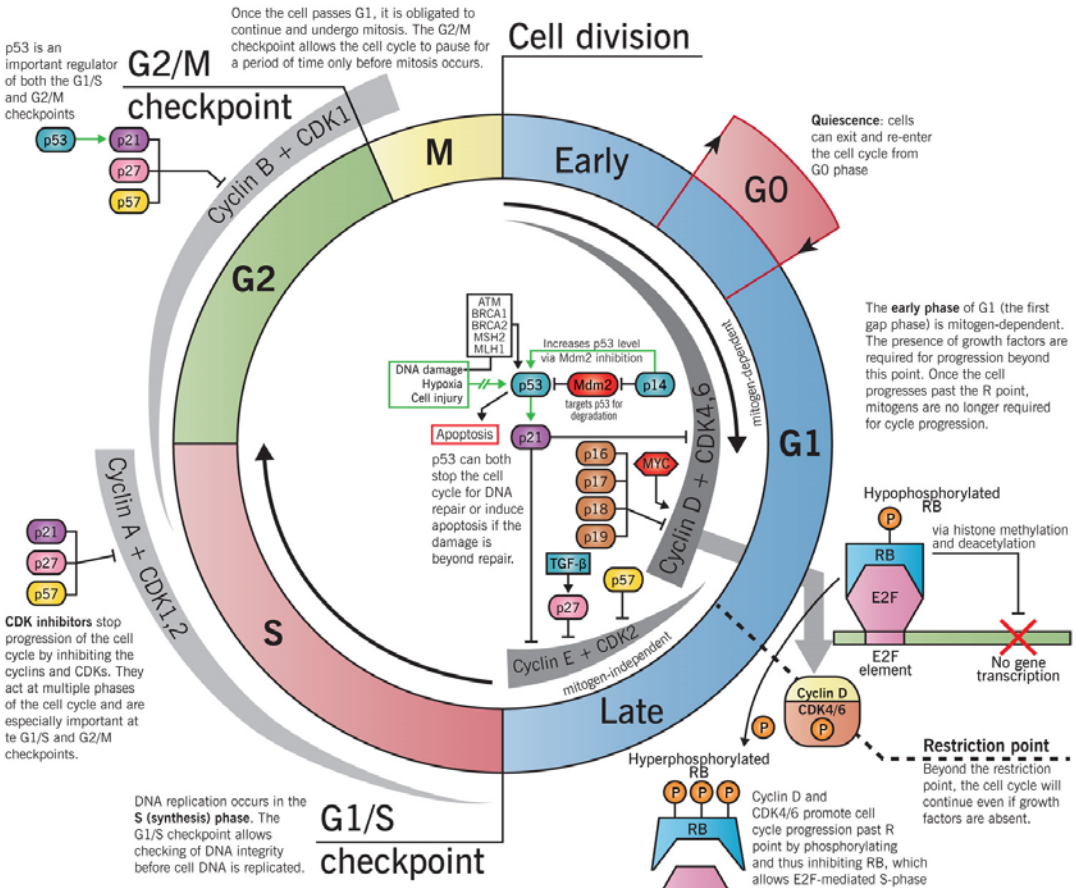
Hallmarks of Cancer



The cell cycle and implications for cancer genetics

Eric Wong

The **cyclins and CDKs** promote cell cycle progression, while the **CDK inhibitors** stop it. The balance between the two groups of molecules determines whether the cell proliferates or is quiescent.



RB

autosomal dominant inheritance

Germline mutations of *RB1* gene causes **retinoblastoma**, an embryonic cancer of the retina. Since RB inhibits cell cycle progression, it is protective against uncontrolled cell proliferation and thus is a **tumour suppressor (ts)**. According to the **Knudson two-hit hypothesis** for recessive ts genes, both alleles of the RB1 gene must be lost for cancer to develop.

In the **hereditary form**, one allele of the *RB1* gene is mutated or deleted in the germ cells. The other allele is lost via a somatic mutation later on. The hereditary form features bilateral, multifocal retinal involvement and typically manifests early, before the age of 1.

Sporadic retinoblastoma is more common, and is often unilateral and unifocal in origin. The age of onset is usually later, between the ages of 1 and 2.

p53

autosomal dominant inheritance

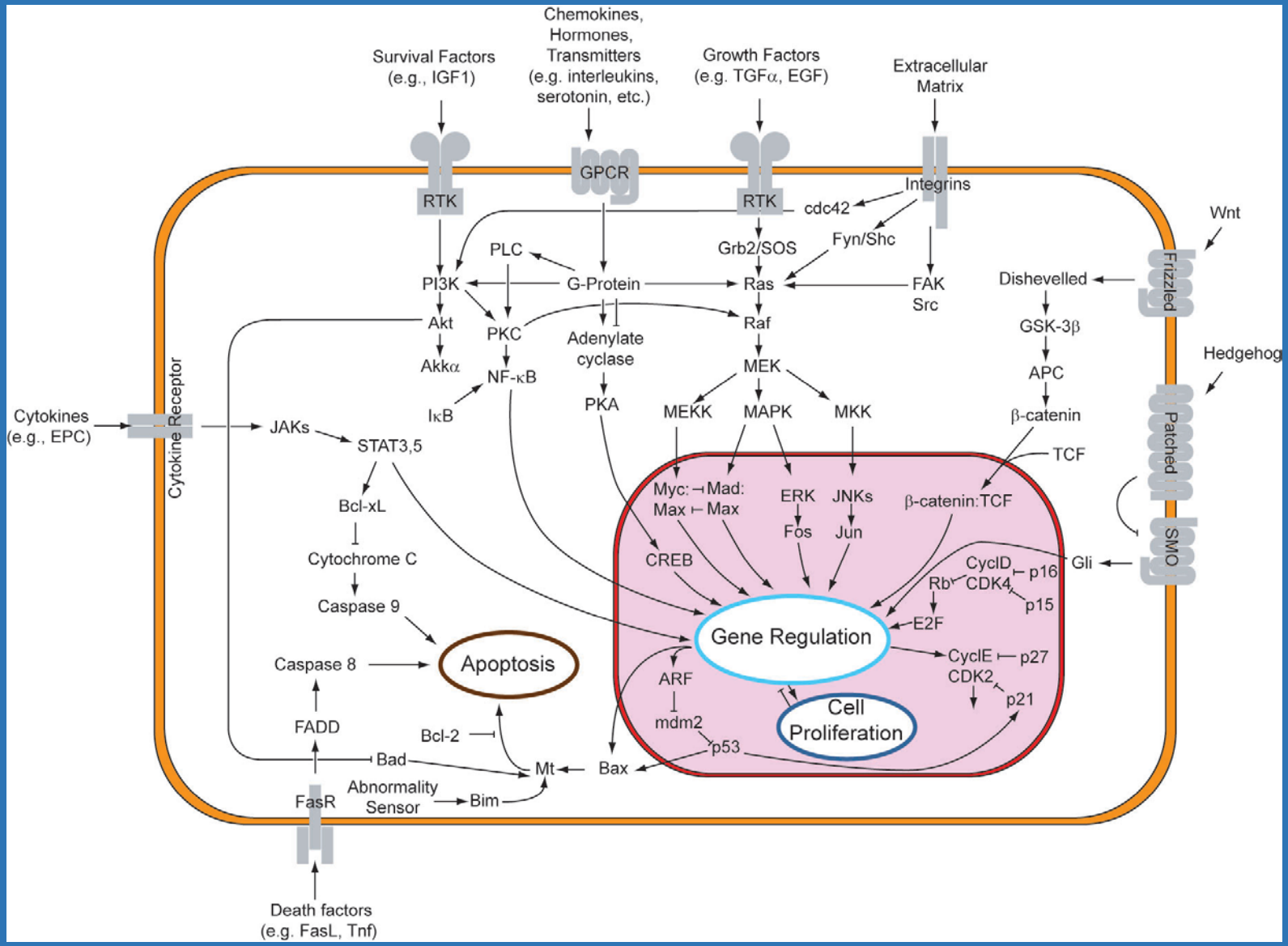
Germline mutations of *p53* gene causes **Li-Fraumeni syndrome**, in which multiple cancers develop at a relatively young age (<45). Breast cancer, osteosarcoma, leukemia, lymphoma, and adrenocortical carcinomas are the most common cancers found in Li-Fraumeni syndrome.

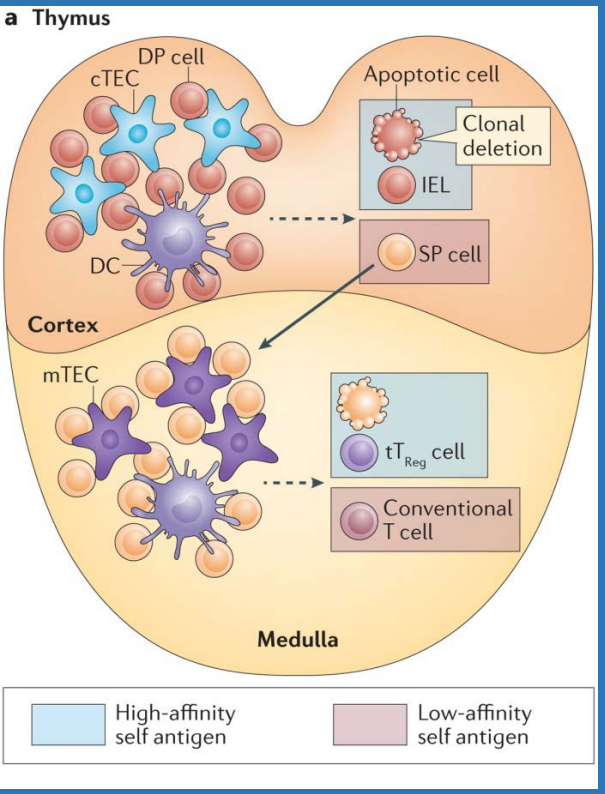
Similar to retinoblastoma, the **two-hit hypothesis** applies to Li-Fraumeni syndrome as well. One allele of *p53* is lost in the germline and the second allele is lost somatically. The risk of cancer is dominantly inherited.

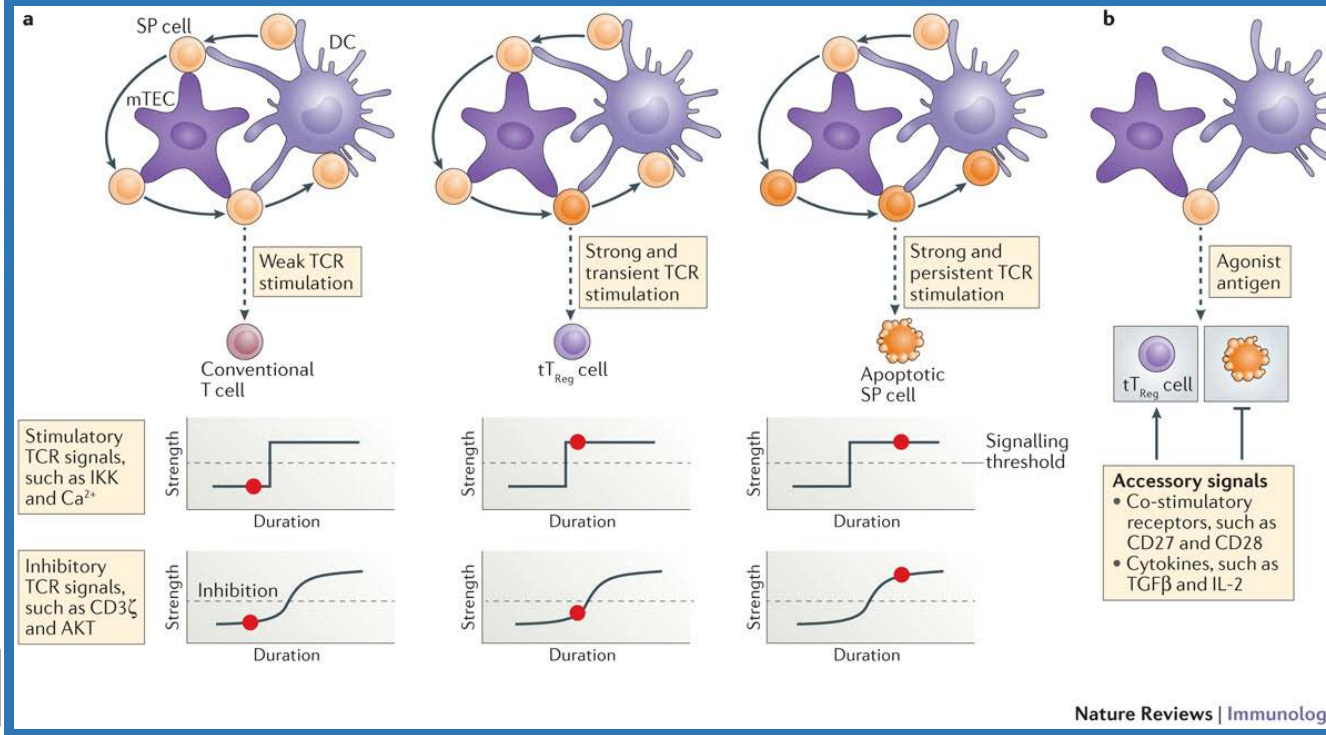
p53 is called the "guardian of the genome." It is lost in almost every type of cancer because its loss confers a significant survival advantage, leading to rapid, unchecked proliferation and evasion of apoptosis.

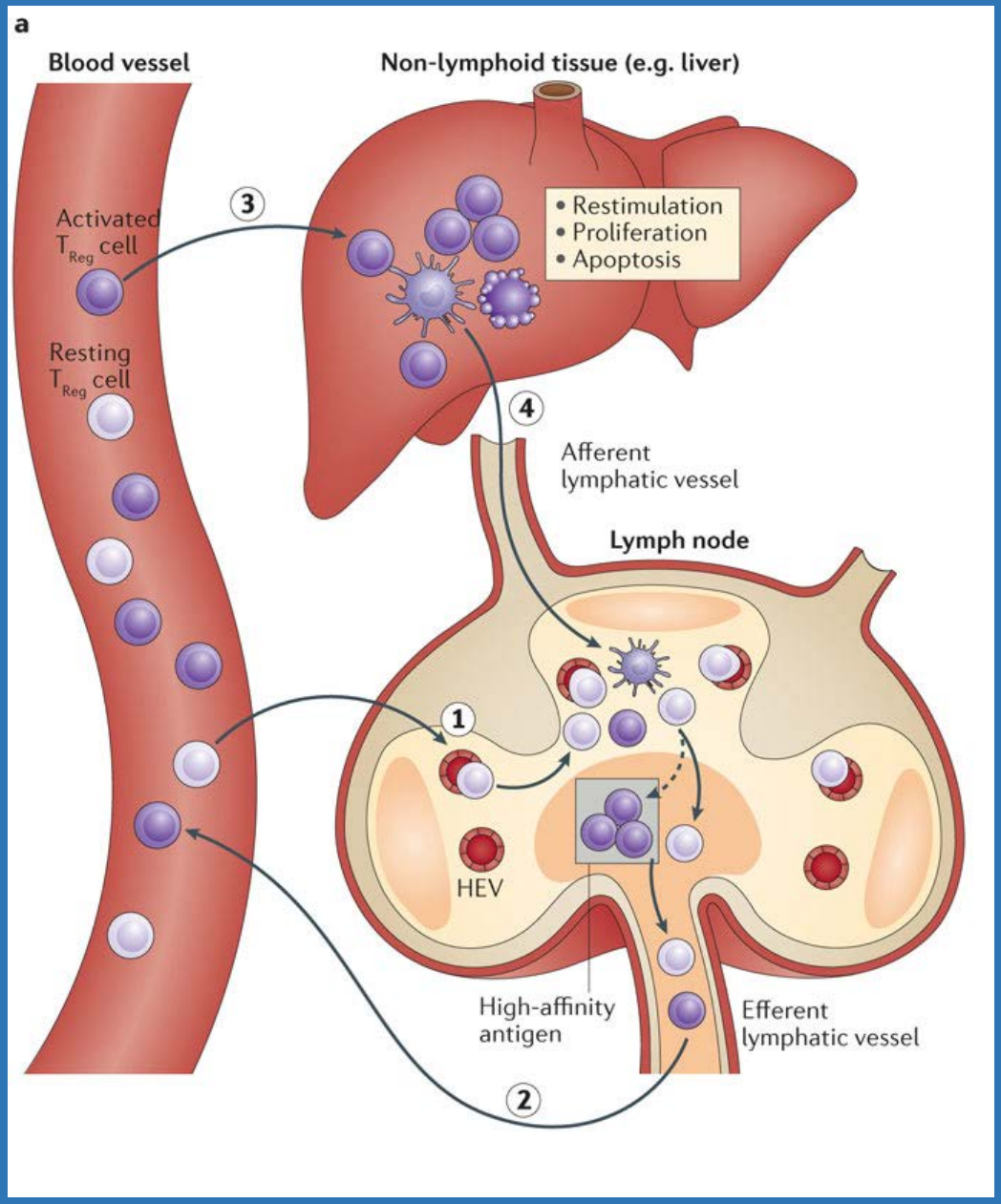
Oncogenes *The lack of inhibition or gain of function of which will lead to cancer.*	Tumour suppressors *The increased inhibition or loss of function of which will lead to cancer.*
Cyclins	RB
Cyclin-dependent kinases (CDKs)	p53
E2F	p21, p27, p57 (CIP/KIP/WAF)
MYC	p14/ARF
Mdm2	p16/INK4a, p17, p18, p19
	TGF-β receptor
	BRCA1, BRCA2
	ATM

Cancer Cell Signaling

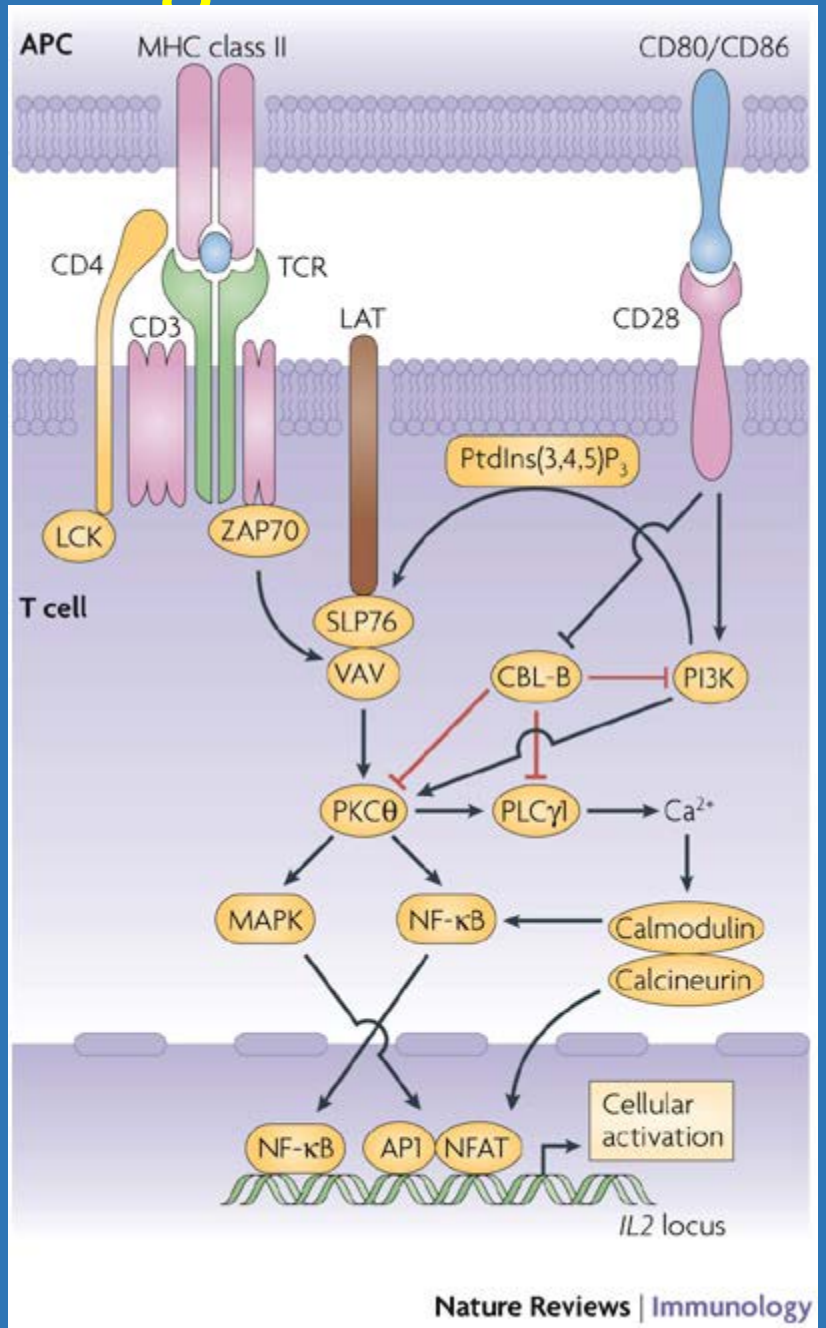



a Thymus
DP cell
cTEC
Apoptotic cell
Clonal deletion
IEL
SP cell
DC
Cortex
mTEC
tT$_{Reg}$ cell
Conventional T cell
Medulla
High-affinity self antigen
Low-affinity self antigen


a
SP cell
DC
mTEC
Weak TCR stimulation
Strong and transient TCR stimulation
Strong and persistent TCR stimulation
Conventional T cell
tT$_{Reg}$ cell
Apoptotic SP cell
Stimulatory TCR signals, such as IKK and Ca^{2+}
Inhibitory TCR signals, such as CD3ζ and AKT
Strength
Duration
Inhibition
Signalling threshold
b
Agonist antigen
tT$_{Reg}$ cell
Accessory signals
• Co-stimulatory receptors, such as CD27 and CD28
• Cytokines, such as TGFβ and IL-2
Nature Reviews | Immunolog

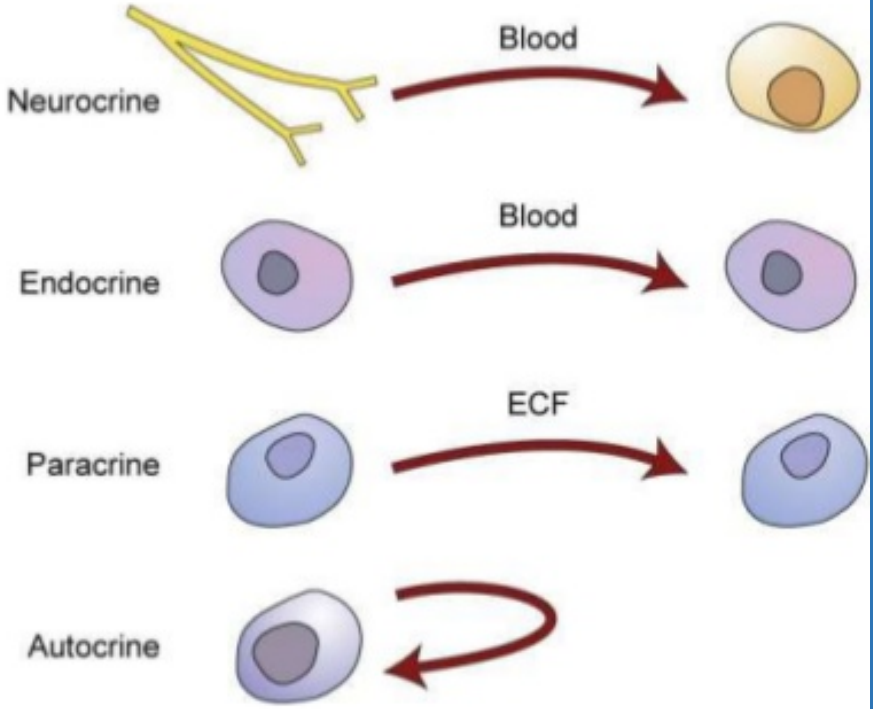

a
Blood vessel
Non-lymphoid tissue (e.g. liver)
Activated T_{Reg} cell
Resting T_{Reg} cell
3
• Restimulation
• Proliferation
• Apoptosis
4
Afferent lymphatic vessel
Lymph node
1
HEV
High-affinity antigen
Efferent lymphatic vessel
2

T-Cell Signal Transduction

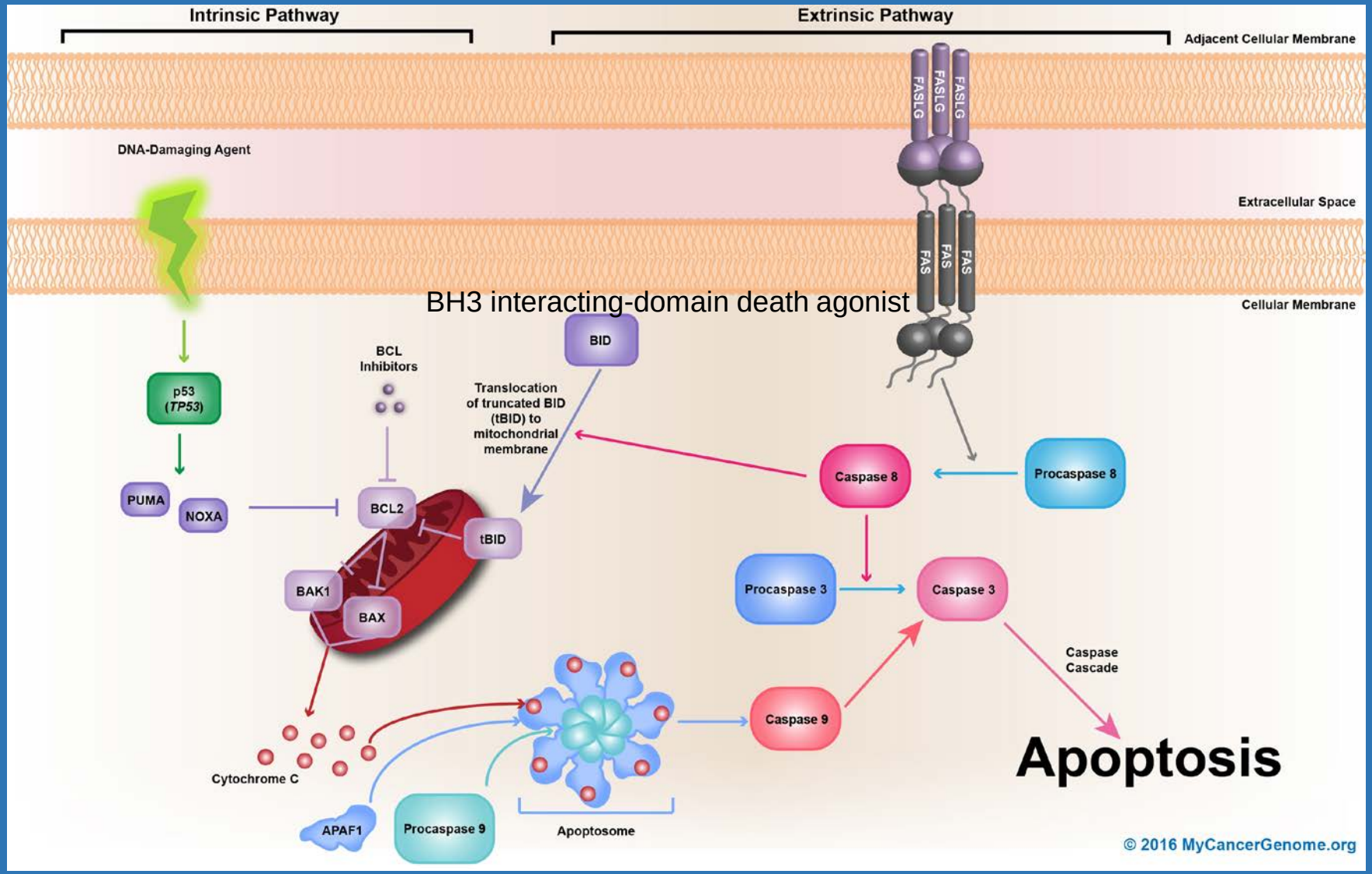


Hormones

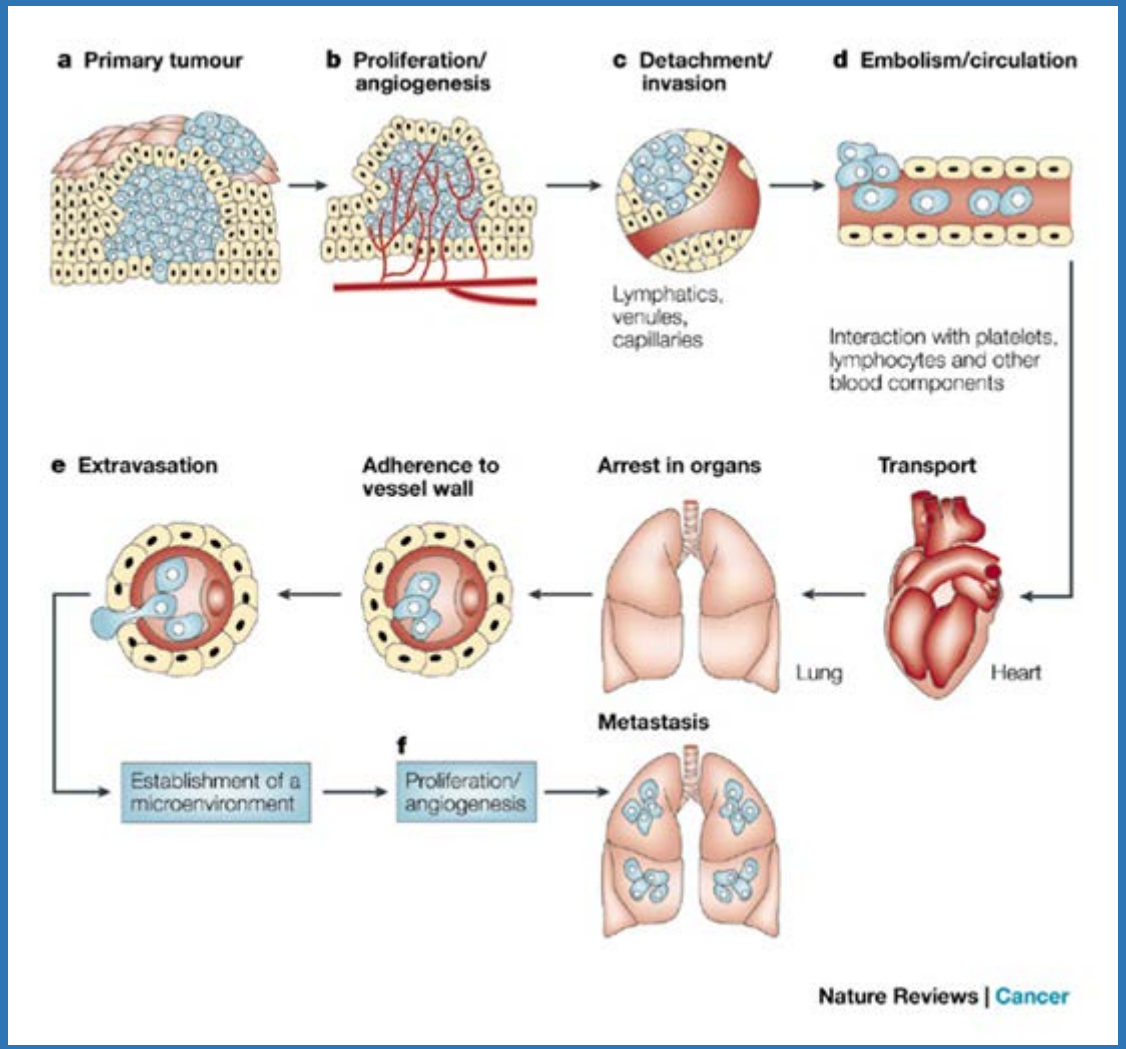
- **Neurocrine**—secretion of hormones into the bloodstream by neurons
- **Endocrine**—secretion of hormones into the bloodstream by endocrine glands
- **Paracrine**—hormone molecule secreted by one cell affects adjacent cells
- **Autocrine**—hormone molecule secreted by a cell affects the secreting cell



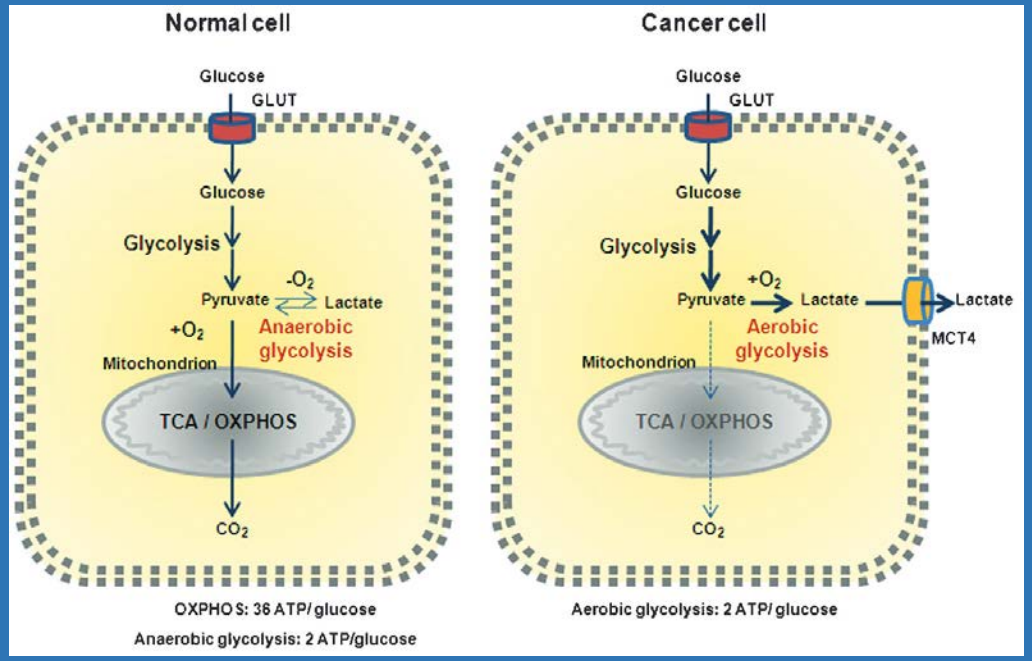
APOPTOSIS

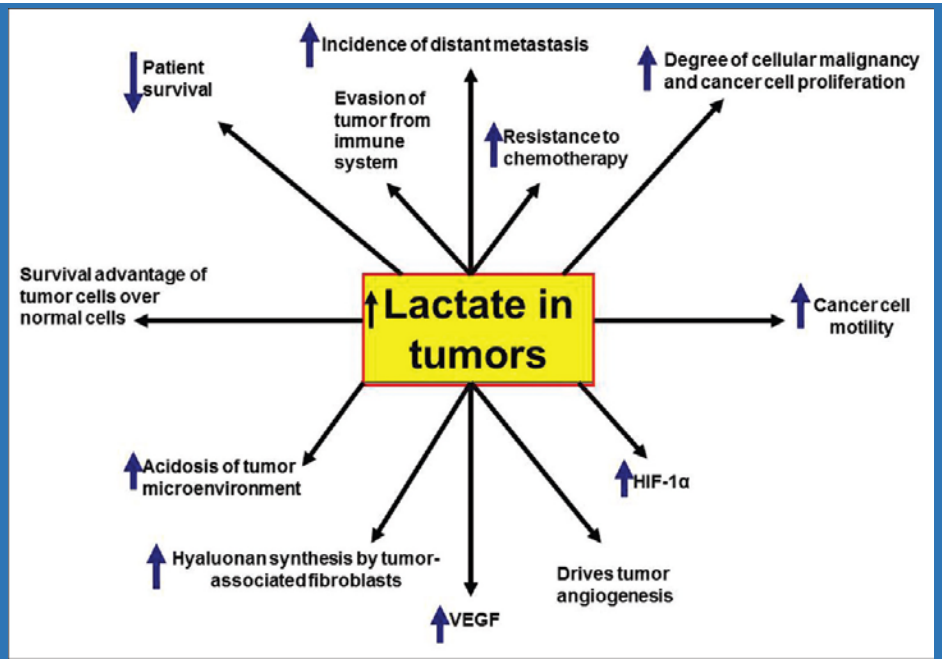

Intrinsic Pathway
Extrinsic Pathway
Adjacent Cellular Membrane
FASLG
FASLG
FASLG
DNA-Damaging Agent
Extracellular Space
FAS
FAS
FAS
Cellular Membrane
BH3 interacting-domain death agonist
BID
BCL
Inhibitors
p53
(TP53)
Translocation
of truncated BID
(tBID) to
mitochondrial
membrane
Caspase 8
Procaspase 8
PUMA
NOXA
BCL2
tBID
BAK1
BAX
Procaspase 3
Caspase 3
Caspase
Cascade
Caspase 9
Cytochrome C
APAF1
Procaspase 9
Apoptosome
Apoptosis
© 2016 MyCancerGenome.org

Invasion Metastasis Cascade

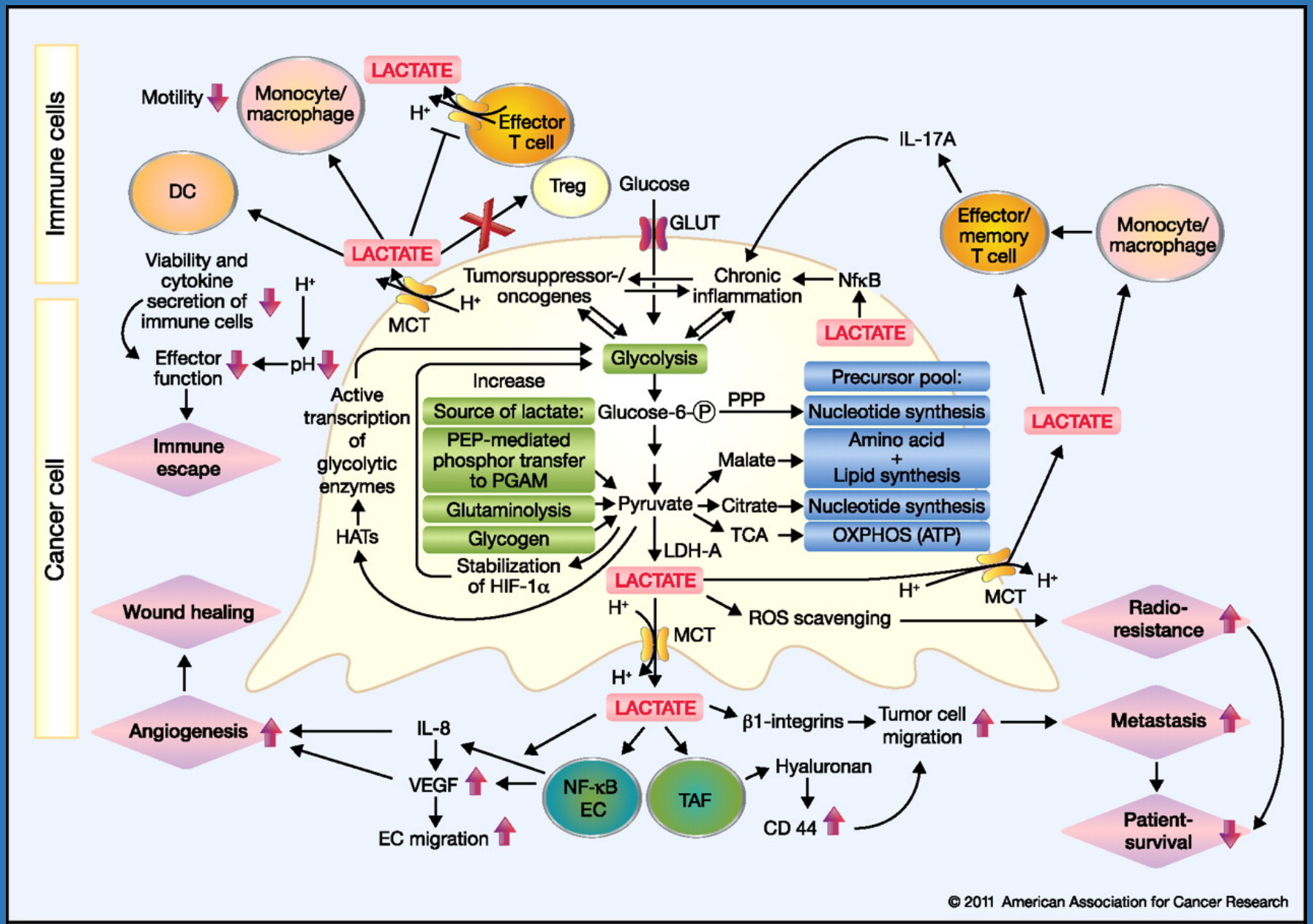


Cancer Cell Metabolism

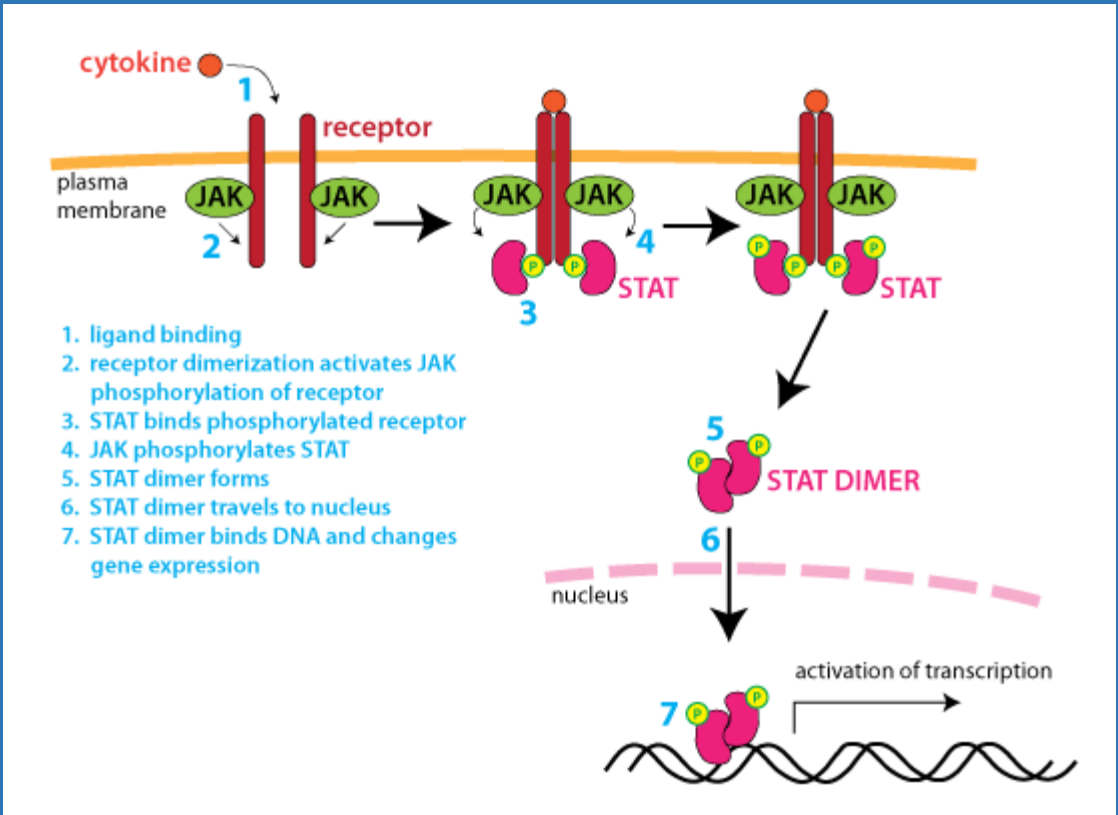




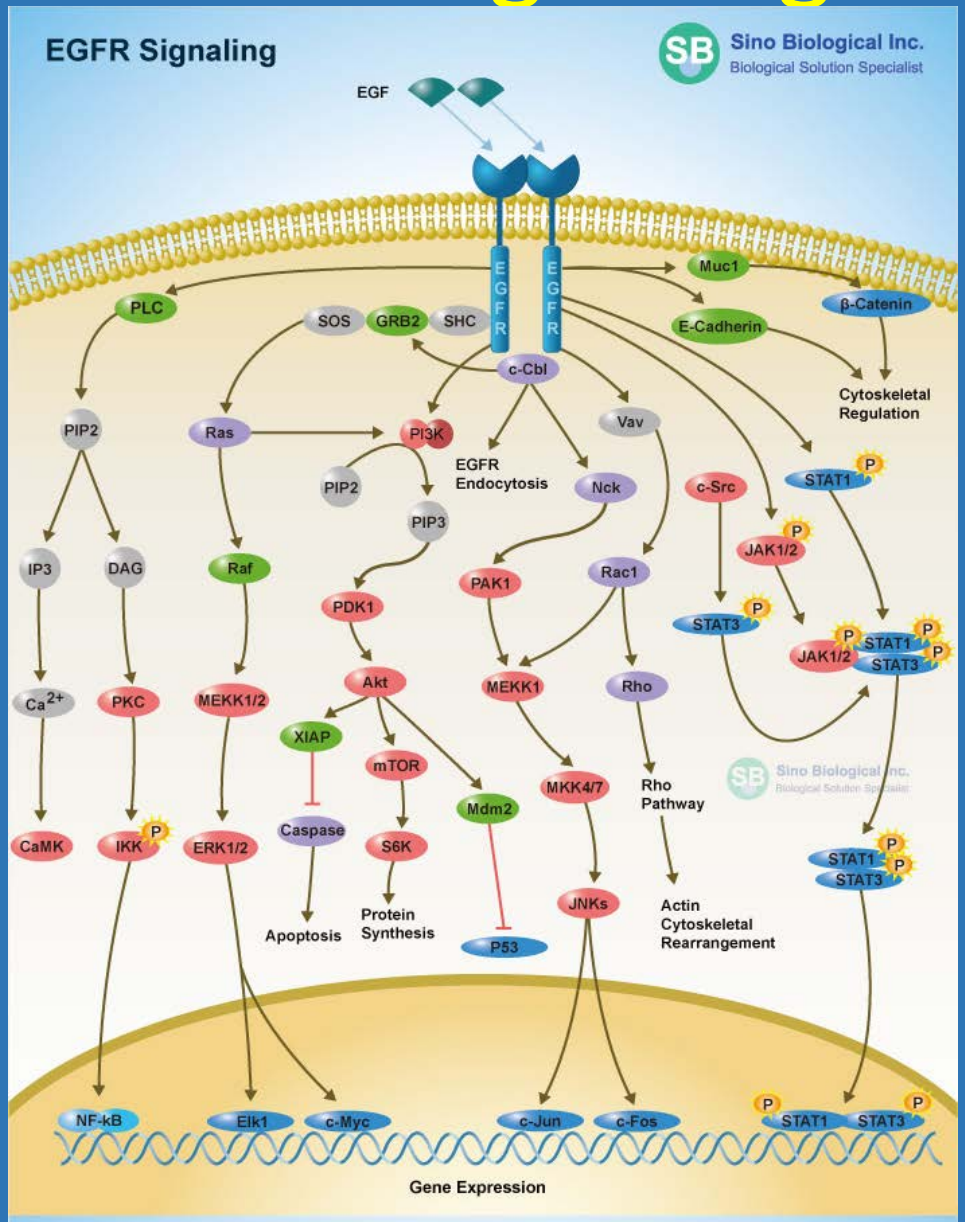
Cancer Cell Metabolism



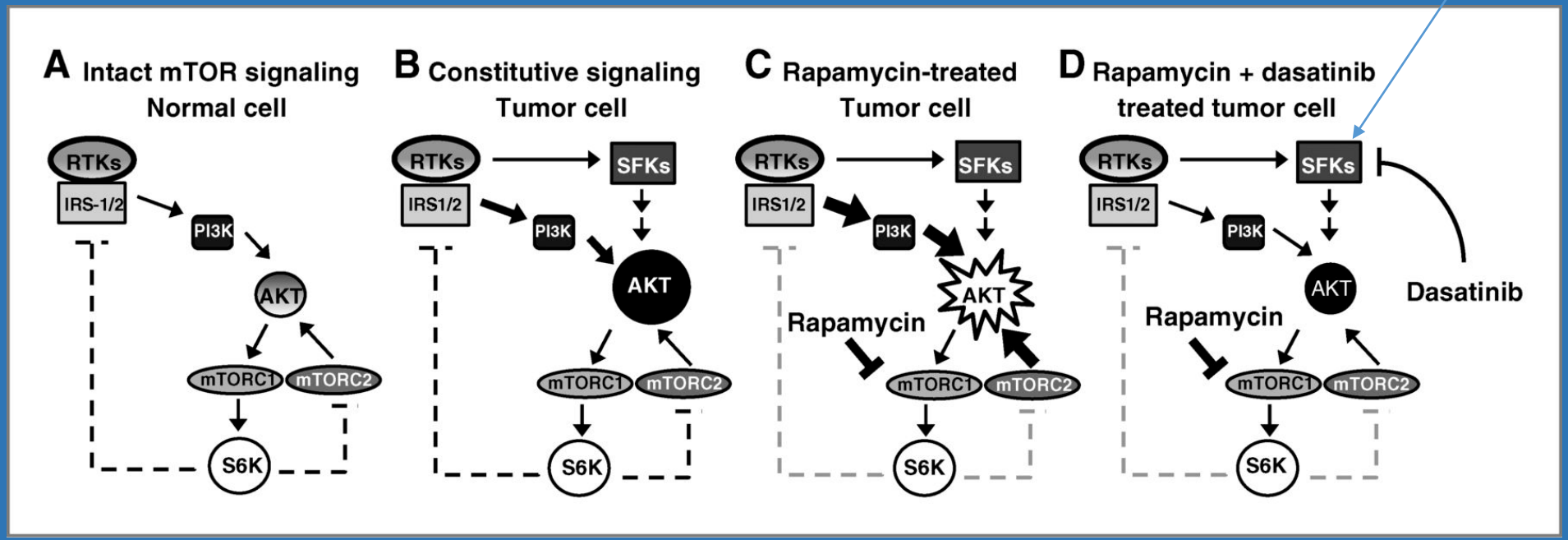
Jak/Stat Signalling



EGFR Signaling



Src Signaling



IHC

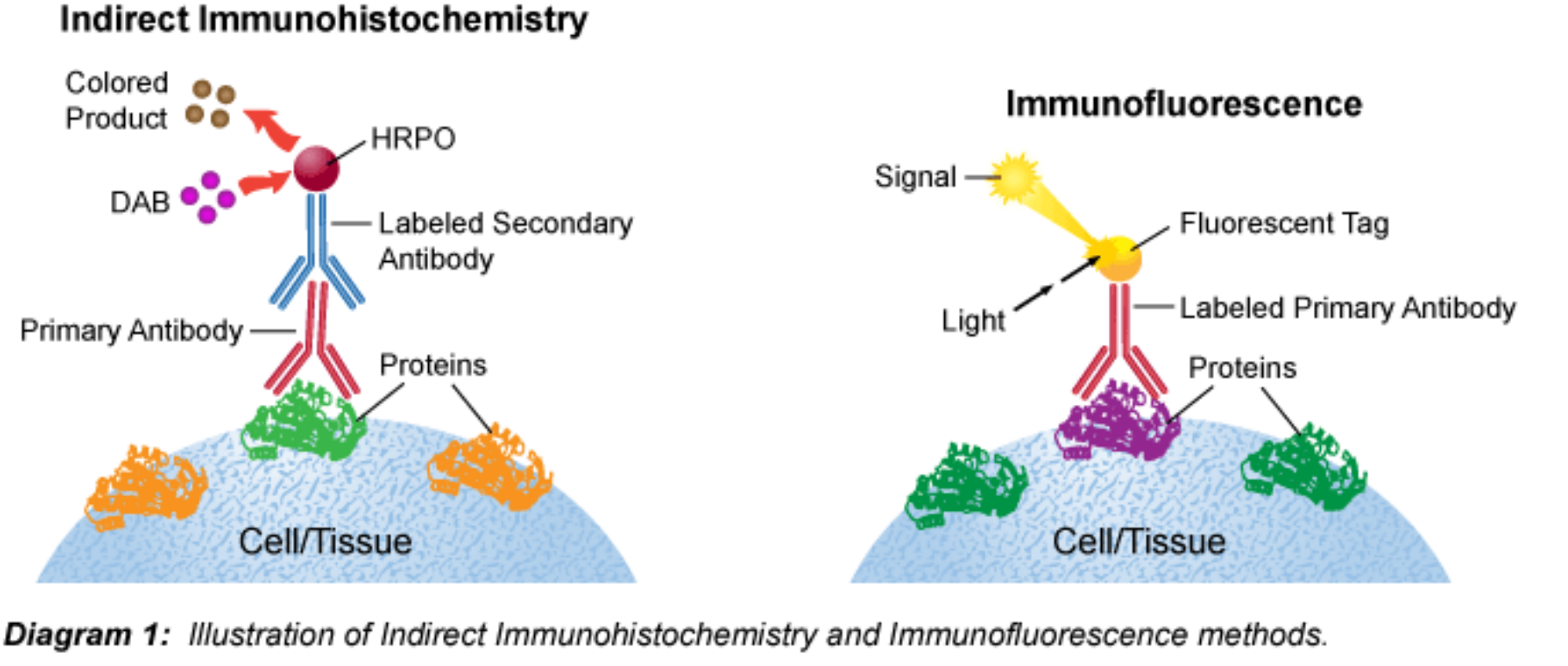
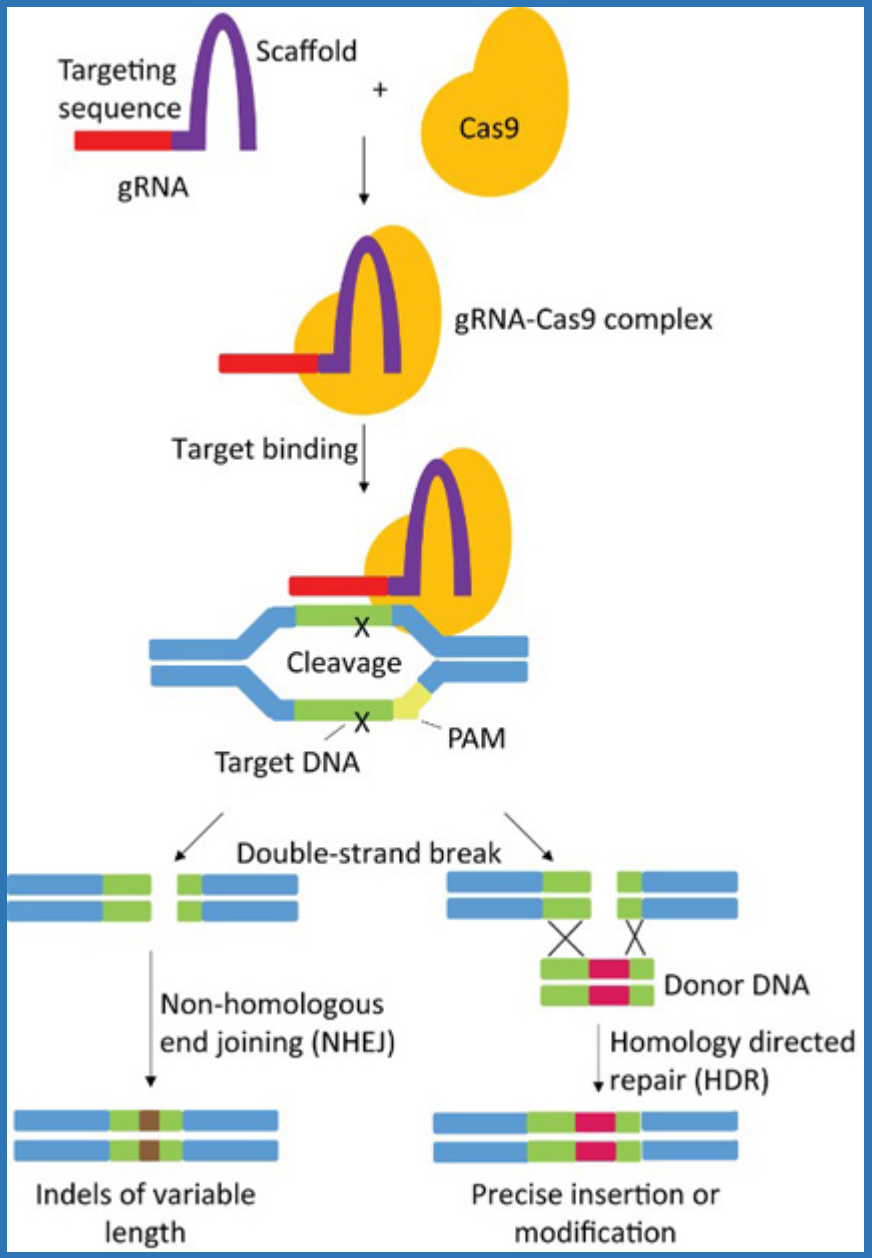


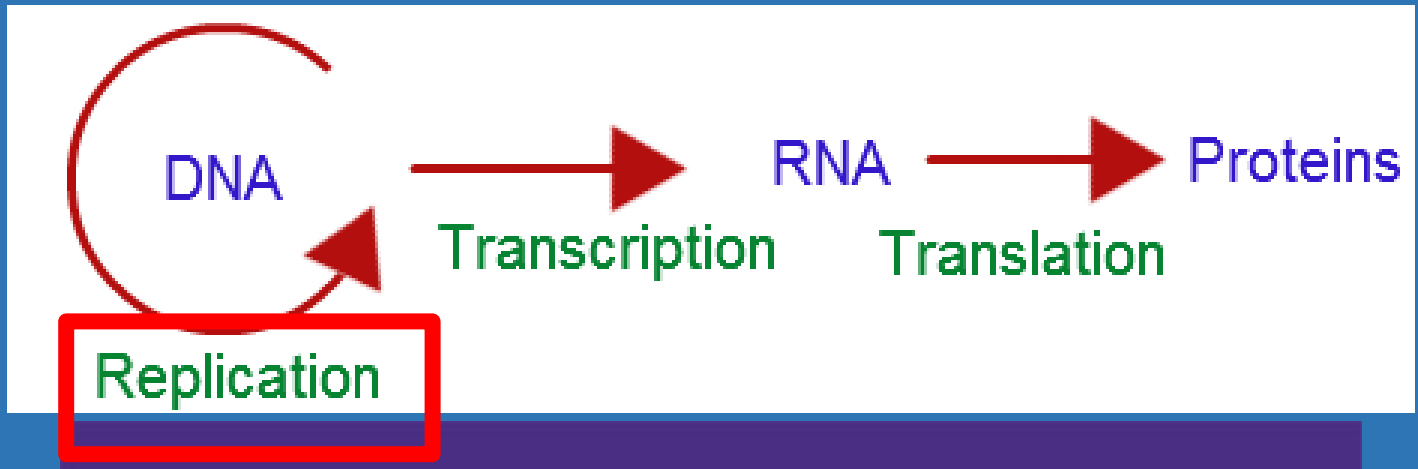
Diagram 1: *Illustration of Indirect Immunohistochemistry and Immunofluorescence methods.*

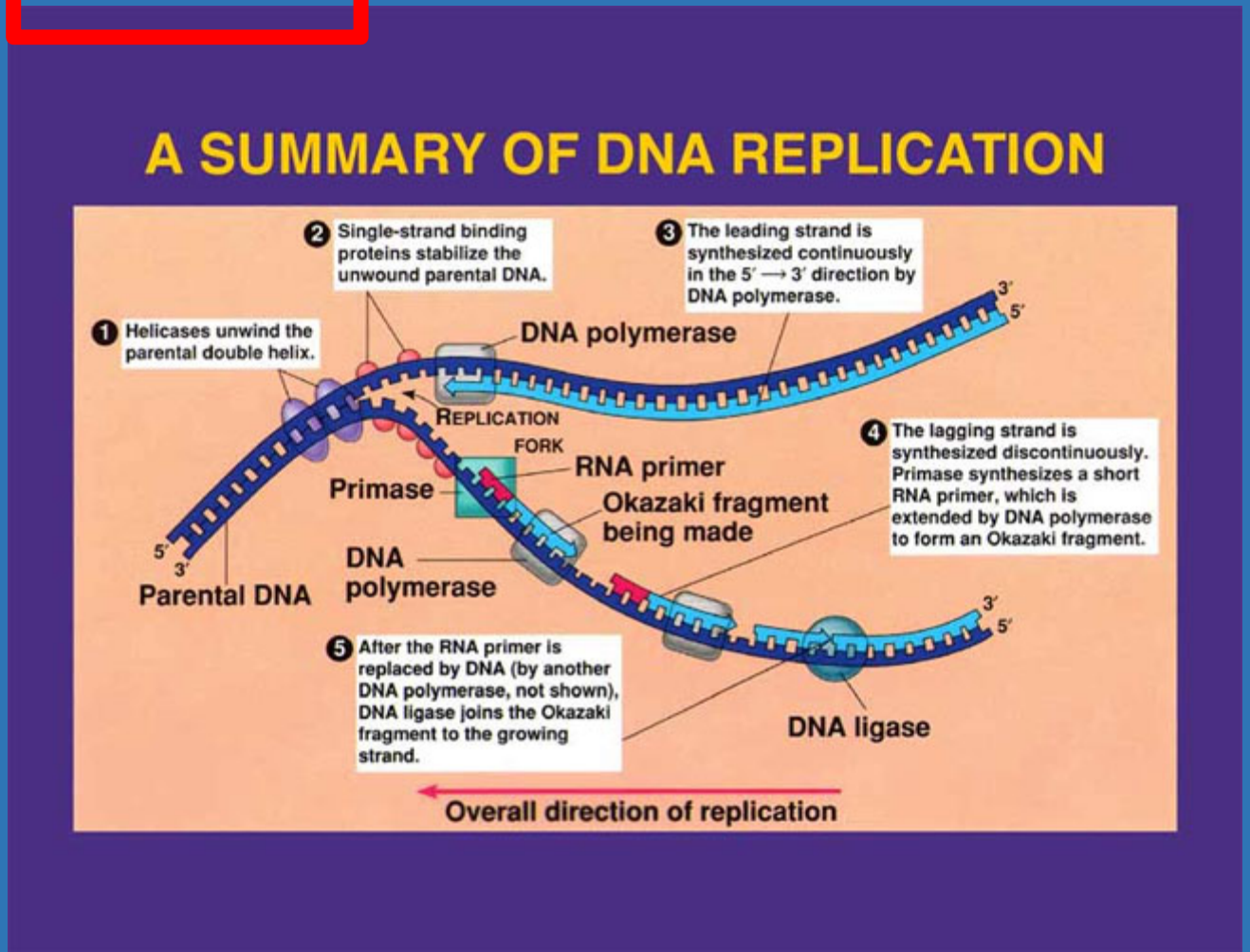
CRISPR/Cas9

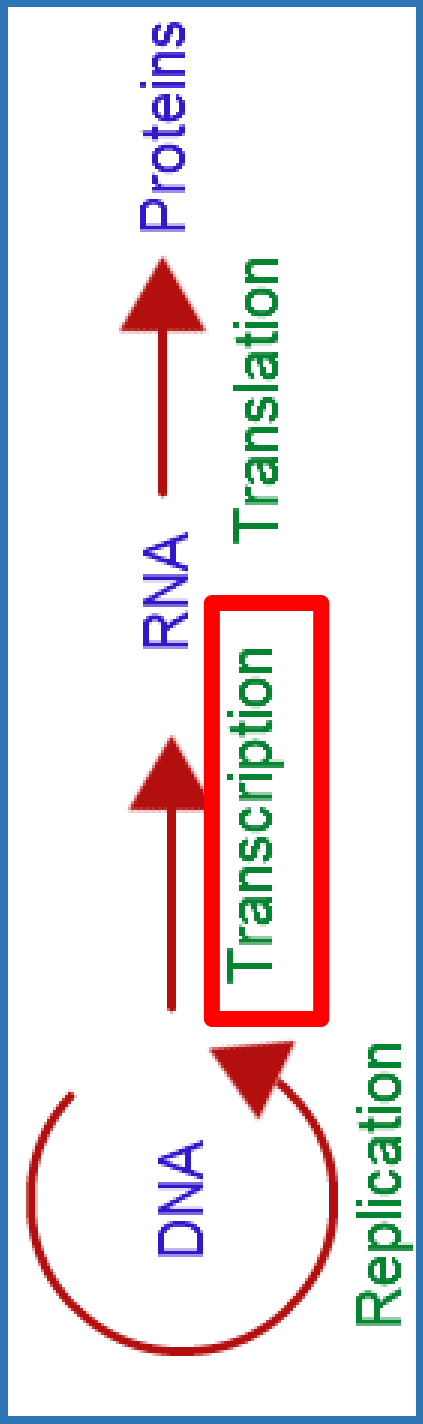

Targeting sequence
Scaffold
gRNA
+
Cas9
gRNA-Cas9 complex
Target binding
X
Cleavage
X
PAM
Target DNA
Double-strand break
Donor DNA
Non-homologous end joining (NHEJ)
Homology directed repair (HDR)
Indels of variable length
Precise insertion or modification

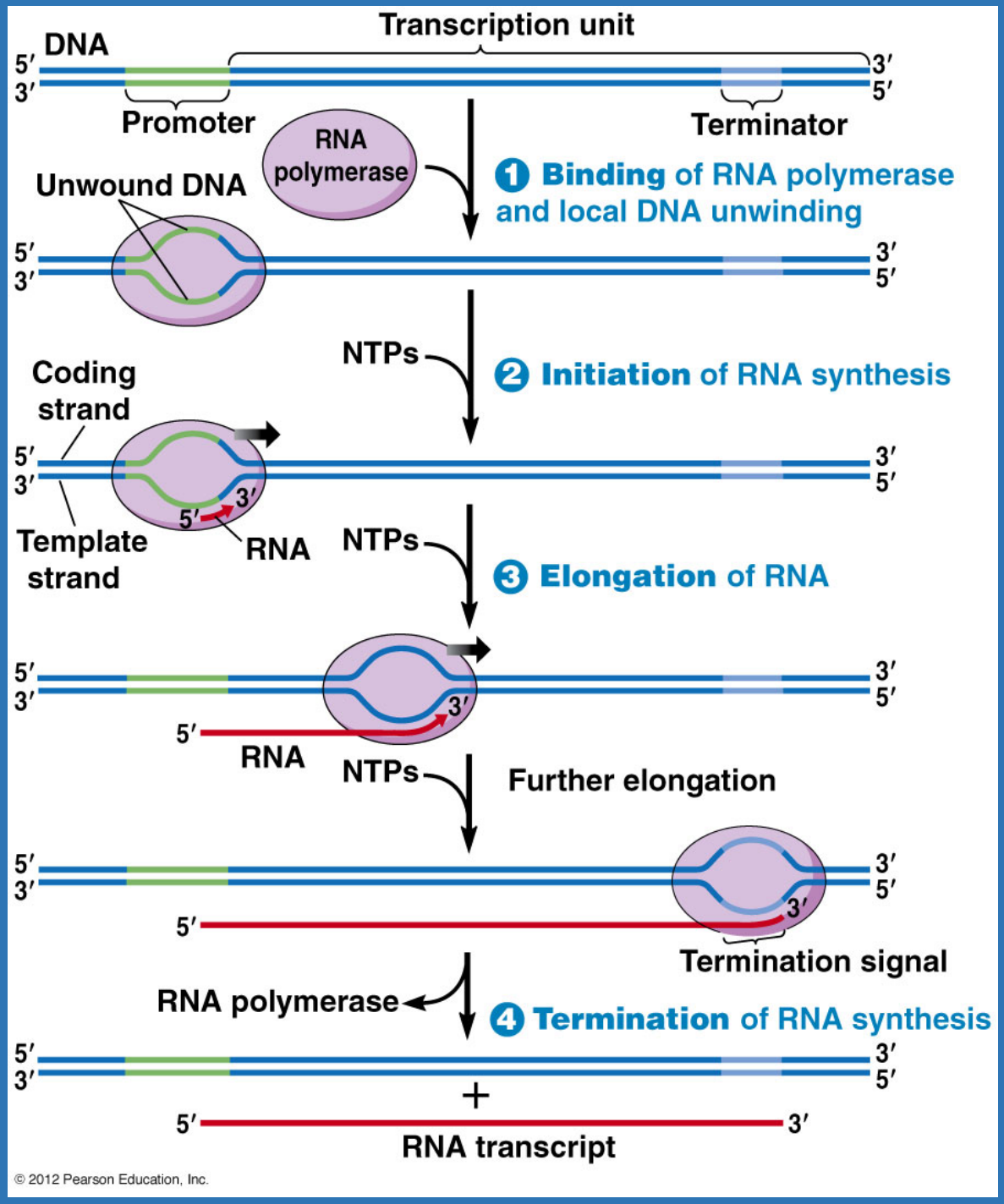
Immunotherapy for NSCLC

- Atezolizumab (aPD-L1), 2016 approval
  - metastatic NSCLC that has progressed during or after first-line chemotherapy with a platinum-based drug.
  - 12.6 vs. 9.7 (docetaxel)
  - Side effects were less frequent
- Pembrolizumab (aPD-L1), 2016 approval
  - the first-line treatment in PD-L1 expressing metastatic NSCLC
  - second-line treatment of metastatic NSCLC (PD-L1 1%+)
  - 10.4 months and 12.7 months vs. 8.5 (docetaxel group)
- Nivolumab (aPD-1,) 2015 approval
  - second-line treatment of NSCLC


DNA
Transcription
RNA
Translation
Proteins
Replication


A SUMMARY OF DNA REPLICATION
1 Helicases unwind the parental double helix.
2 Single-strand binding proteins stabilize the unwound parental DNA.
3 The leading strand is synthesized continuously in the 5′ → 3′ direction by DNA polymerase.
4 The lagging strand is synthesized discontinuously. Primase synthesizes a short RNA primer, which is extended by DNA polymerase to form an Okazaki fragment.
5 After the RNA primer is replaced by DNA (by another DNA polymerase, not shown), DNA ligase joins the Okazaki fragment to the growing strand.
DNA polymerase
REPLICATION FORK
RNA primer
Primase
Okazaki fragment being made
DNA polymerase
Parental DNA
DNA ligase
3′
5′
Overall direction of replication


Proteins
Translation
RNA
Transcription
DNA
Replication


Transcription unit
DNA
5′
3′
3′
5′
Promoter
Terminator
RNA polymerase
Unwound DNA
1 Binding of RNA polymerase and local DNA unwinding
NTPs
2 Initiation of RNA synthesis
Coding strand
Template strand
RNA
3 Elongation of RNA
Further elongation
Termination signal
RNA polymerase
4 Termination of RNA synthesis
RNA transcript
© 2012 Pearson Education, Inc.

1 | A copy of a gene in the DNA is created. The copy, mRNA (messenger RNA), functions as a blueprint for a protein. Instead of thymine (T), uracil (U) is used in mRNA.

2 | The mRNA and the two subunits of the ribosome are assembled.

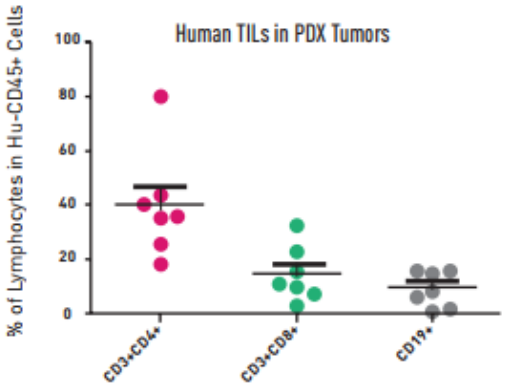
3 | Different tRNA (transport RNA) have different anticodons matching the various codons of mRNA. The amino acid, corresponding to the codon, is connected to the other end of the tRNA.

4 | At the ribosome, tRNA associates with mRNA. The codon and the anticodon have to match, otherwise the tRNA falls off the ribosome.

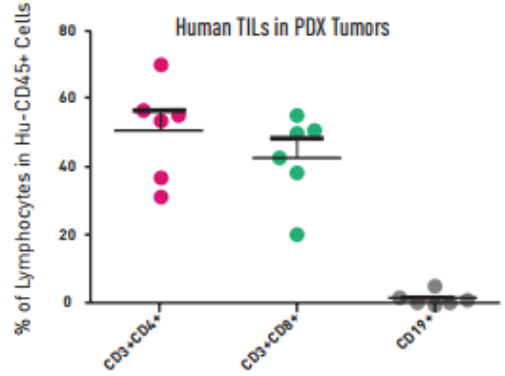
5 | A peptide bond is formed between the amino acids. The growing protein chain is moved to the tRNA in the right hand position. Then the ribosome moves one step along the mRNA-molecule in order for the next tRNA to bind.

6 | The amino acid chain is folded to a protein.

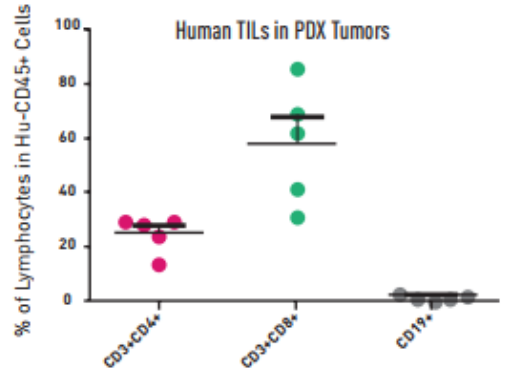
A. Breast



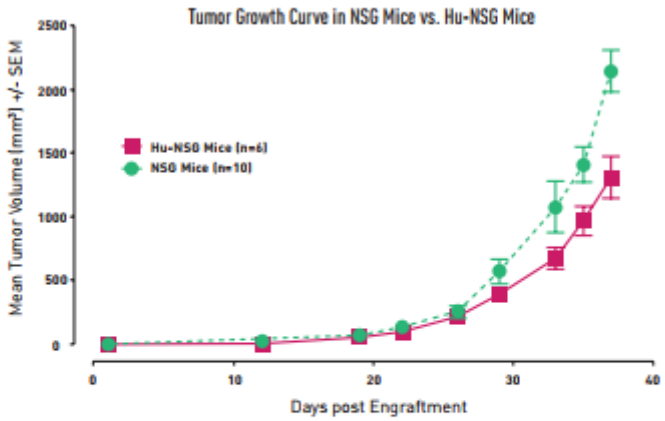
B. Lung



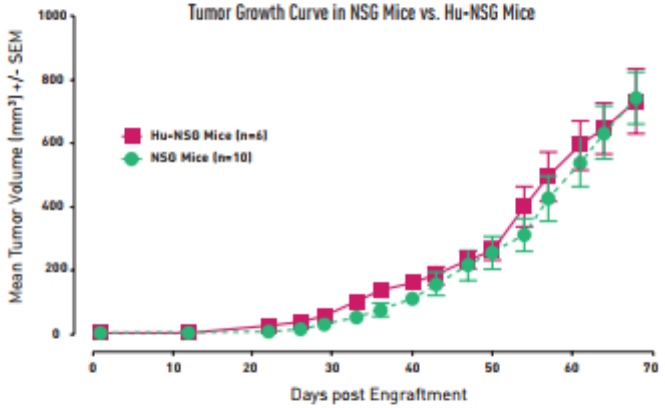
C. Sarcoma



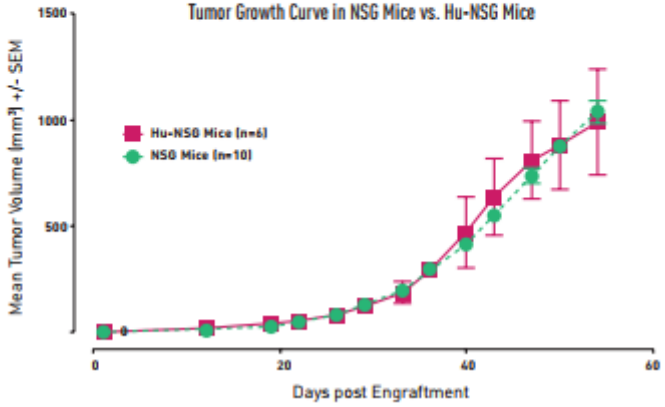
A. Breast

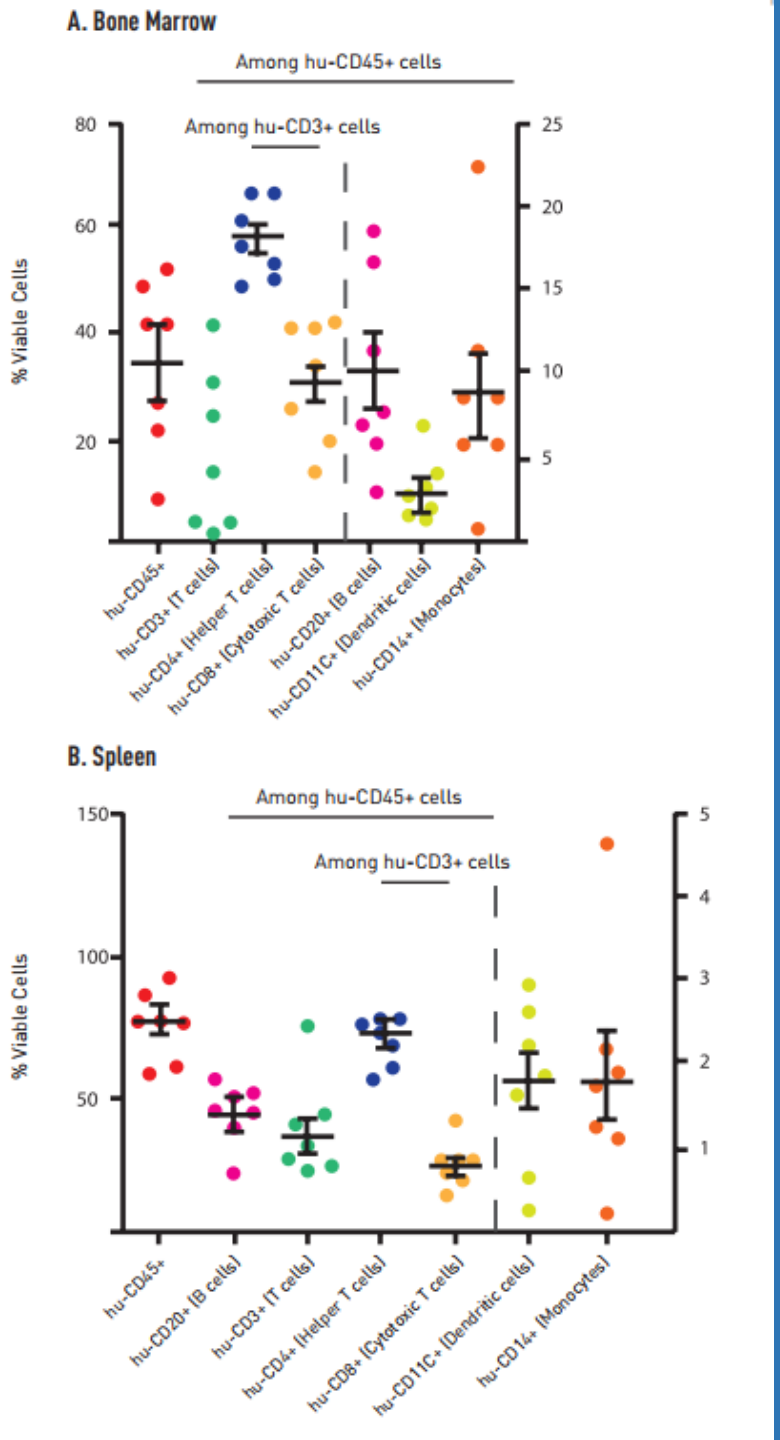


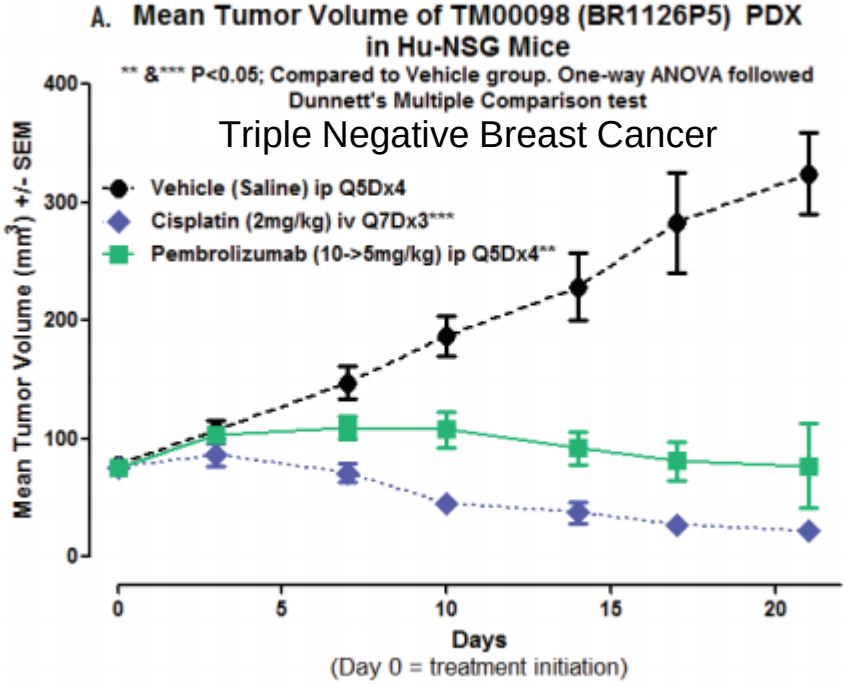
B. Lung

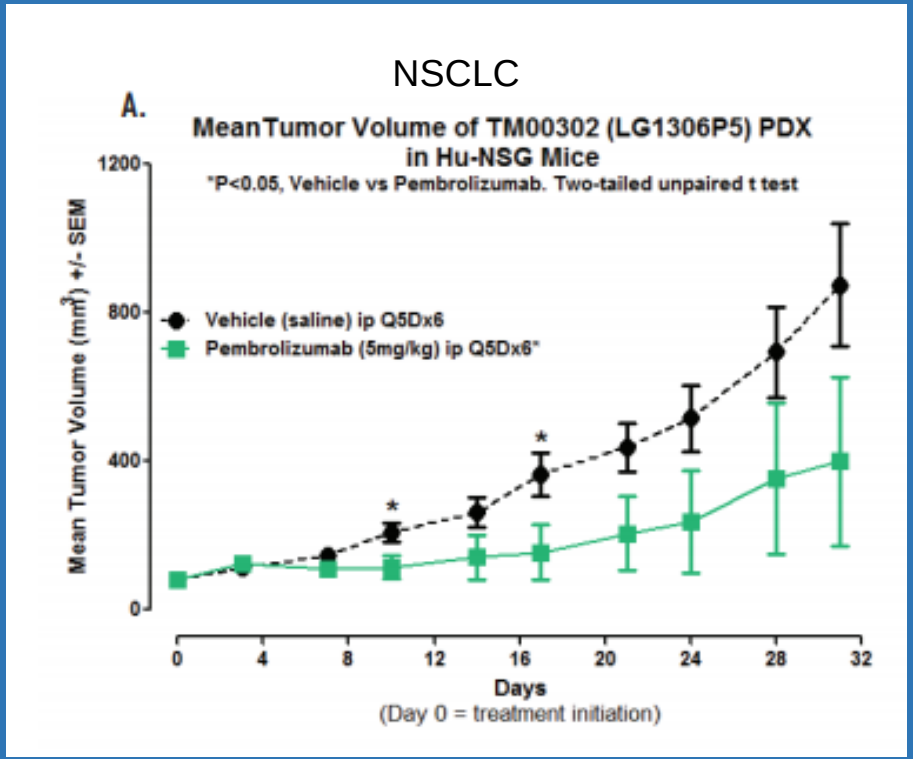


C. Sarcoma

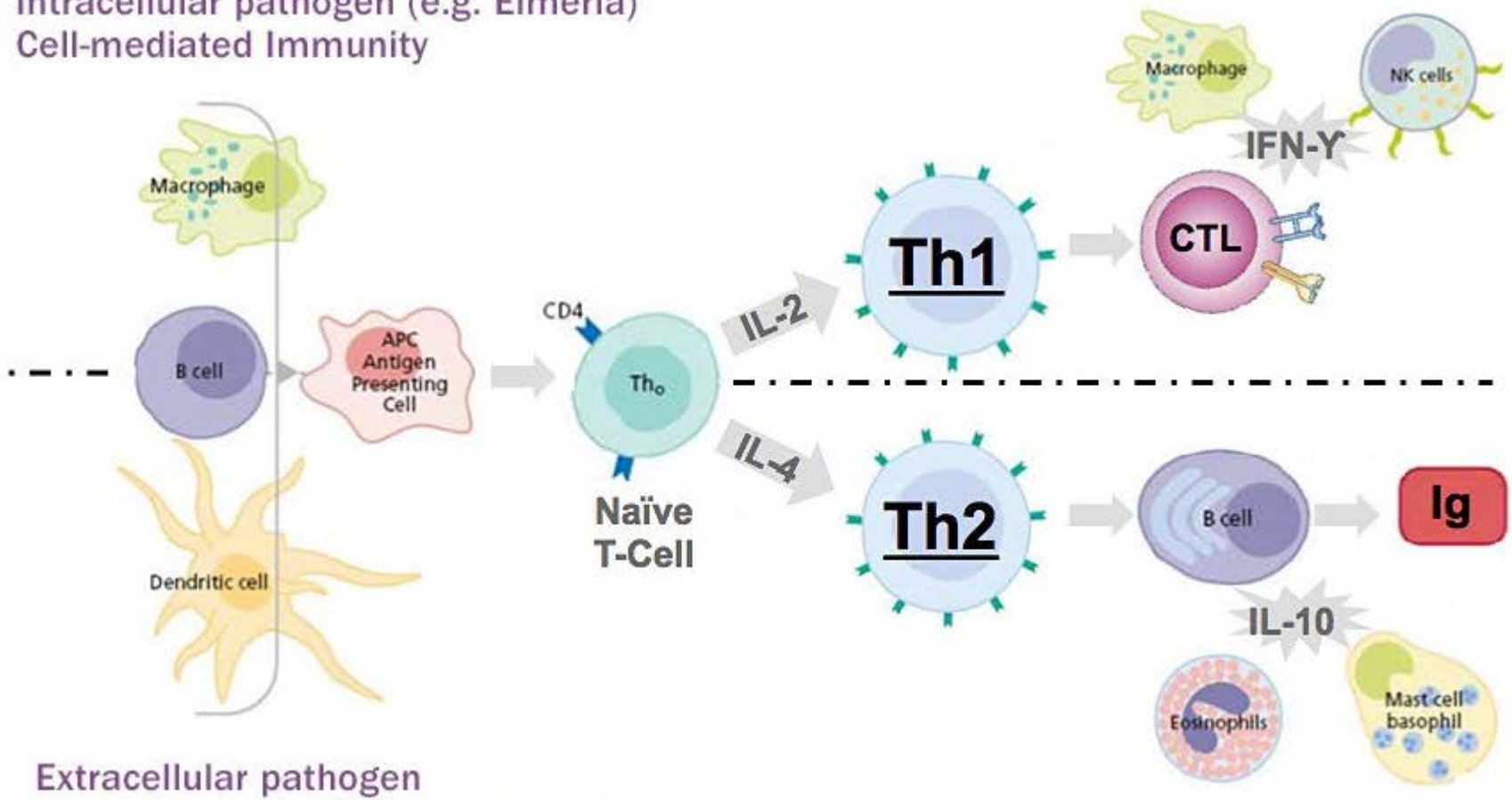



A. Bone Marrow
Among hu-CD45+ cells
Among hu-CD3+ cells
% Viable Cells
80
60
40
20
25
20
15
10
5
hu-CD45+
hu-CD3+ (T cells)
hu-CD4+ (Helper T cells)
hu-CD8+ (Cytotoxic T cells)
hu-CD20+ (B cells)
hu-CD11C+ (Dendritic cells)
hu-CD14+ (Monocytes)
B. Spleen
Among hu-CD45+ cells
Among hu-CD3+ cells
% Viable Cells
150
100
50
5
4
3
2
1
hu-CD45+
hu-CD20+ (B cells)
hu-CD3+ (T cells)
hu-CD4+ (Helper T cells)
hu-CD8+ (Cytotoxic T cells)
hu-CD11C+ (Dendritic cells)
hu-CD14+ (Monocytes)

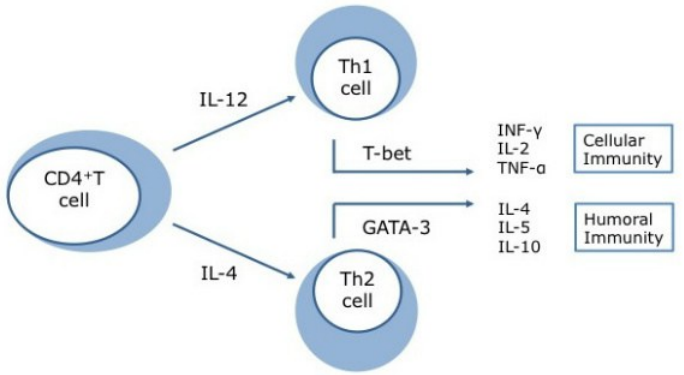

A. Mean Tumor Volume of TM00098 (BR1126P5) PDX in Hu-NSG Mice
** &*** P<0.05; Compared to Vehicle group. One-way ANOVA followed Dunnett's Multiple Comparison test
Triple Negative Breast Cancer
Vehicle (Saline) ip Q5Dx4
Cisplatin (2mg/kg) iv Q7Dx3***
Pembrolizumab (10->5mg/kg) ip Q5Dx4**
Mean Tumor Volume (mm3) +/- SEM
400
300
200
100
0
0
5
10
15
20
Days
(Day 0 = treatment initiation)


NSCLC
A.
MeanTumor Volume of TM00302 (LG1306P5) PDX in Hu-NSG Mice
*P<0.05, Vehicle vs Pembrolizumab. Two-tailed unpaired t test
Vehicle (saline) ip Q5Dx6
Pembrolizumab (5mg/kg) ip Q5Dx6*
Mean Tumor Volume (mm3) +/- SEM
1200
800
400
0
0
4
8
12
16
20
24
28
32
Days
(Day 0 = treatment initiation)

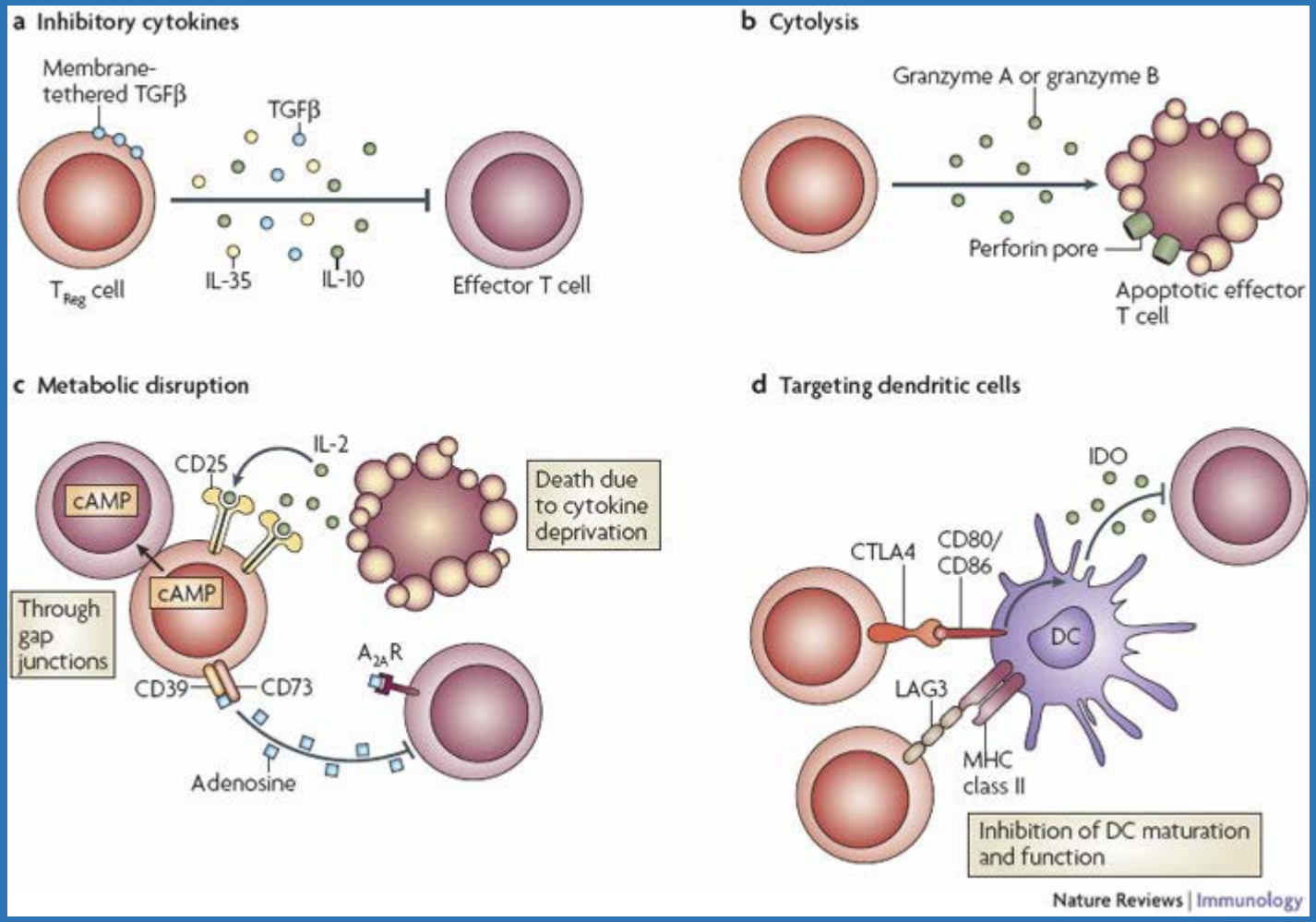
Intracellular pathogen (e.g. Eimeria)
Cell-mediated Immunity



Extracellular pathogen
Humoral immunity (antibodies = Ig)



Regulatory T-Cells



DISTRIBUTION OF Ig ISOTYPES

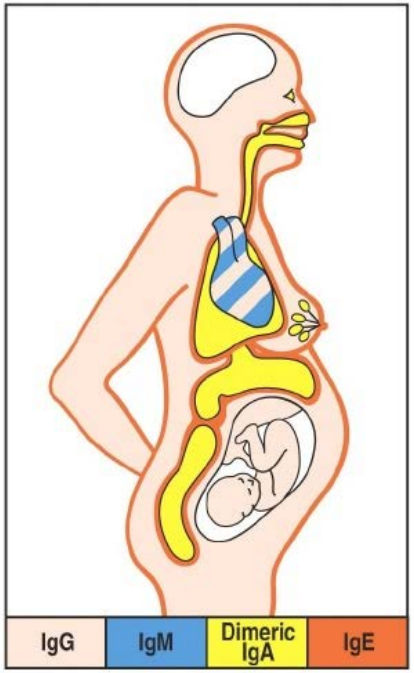
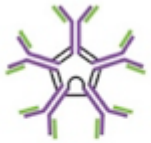
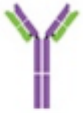
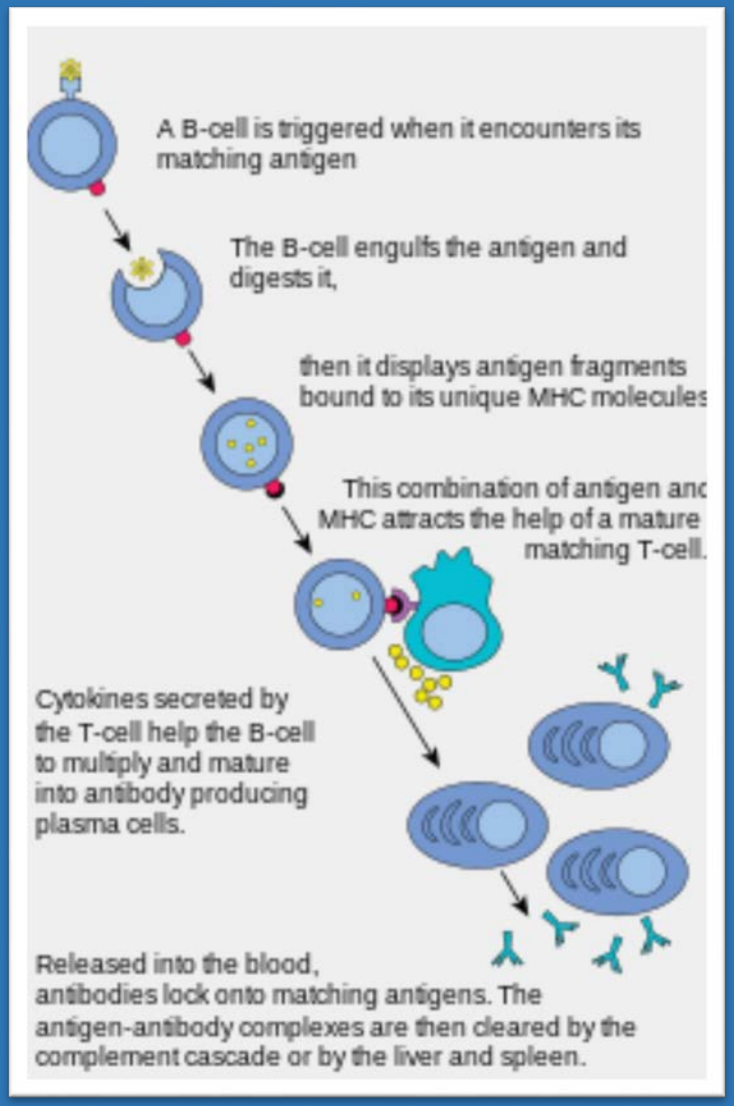


Figure 9-22 Immunobiology, 6/e. (© Garland Science 2005)

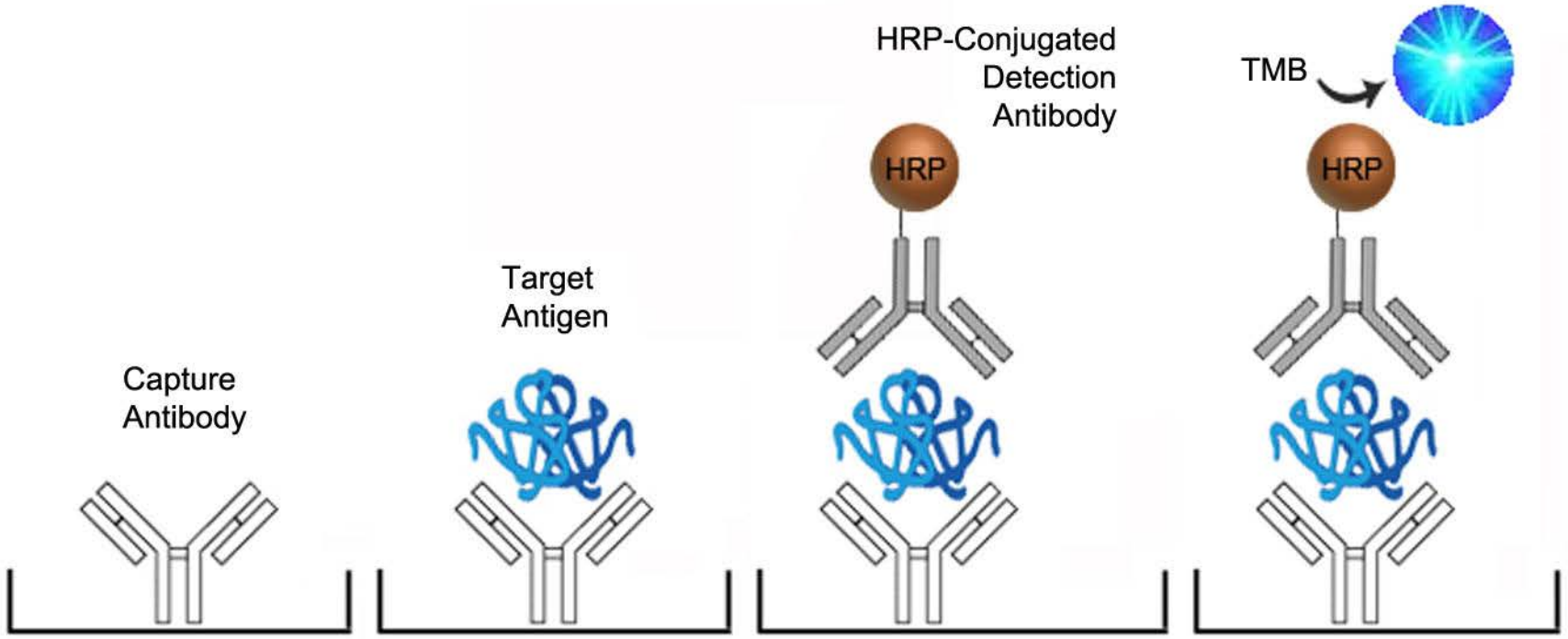
Fig. 9-22

			 Secretory component		
	IgM	IgG	IgA	IgE	IgD
Heavy Chain	μ (mu)	γ (gamma)	α (alpha)	ε (epsilon)	δ (delta)
MW (Da)	900k	150k	385k	200k	180k
% of total antibody in serum	6%	80%	13%	0.002%	1%
Fixes complement	Yes	Yes	No	No	No
Function	Primary response, fixes complement. Monomer serves as B-cell receptor	Main blood antibody, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva	Antibody of allergy and anti-parasitic activity	B cell Receptor

B-Cells


A B-cell is triggered when it encounters its matching antigen
The B-cell engulfs the antigen and digests it,
then it displays antigen fragments bound to its unique MHC molecules
This combination of antigen and MHC attracts the help of a mature matching T-cell.
Cytokines secreted by the T-cell help the B-cell to multiply and mature into antibody producing plasma cells.
Released into the blood, antibodies lock onto matching antigens. The antigen-antibody complexes are then cleared by the complement cascade or by the liver and spleen.

Sandwich ELISA (enzyme-linked immunosorbent assay)



LegendPLEX Cytokine Profiling