



The Role of HHLA2 in Non-Small Cell Lung Cancer


Name of student
Off-Topic Candidacy Exam
Date of Exam

Advisor: Name of Advisor

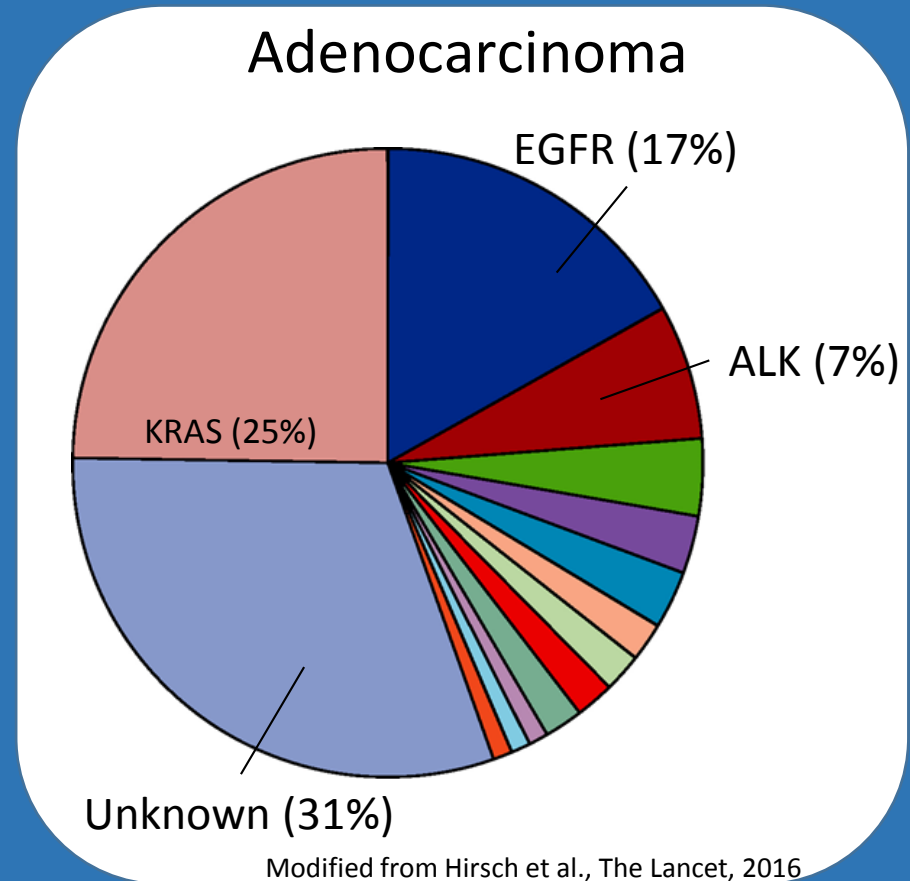
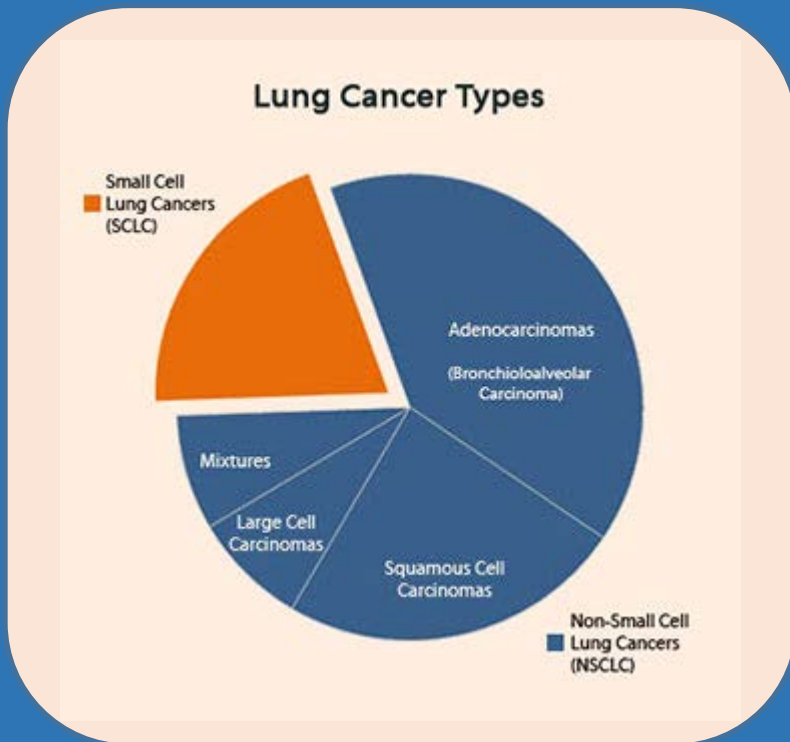
Lung Cancer Statistics

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

Estimated Deaths

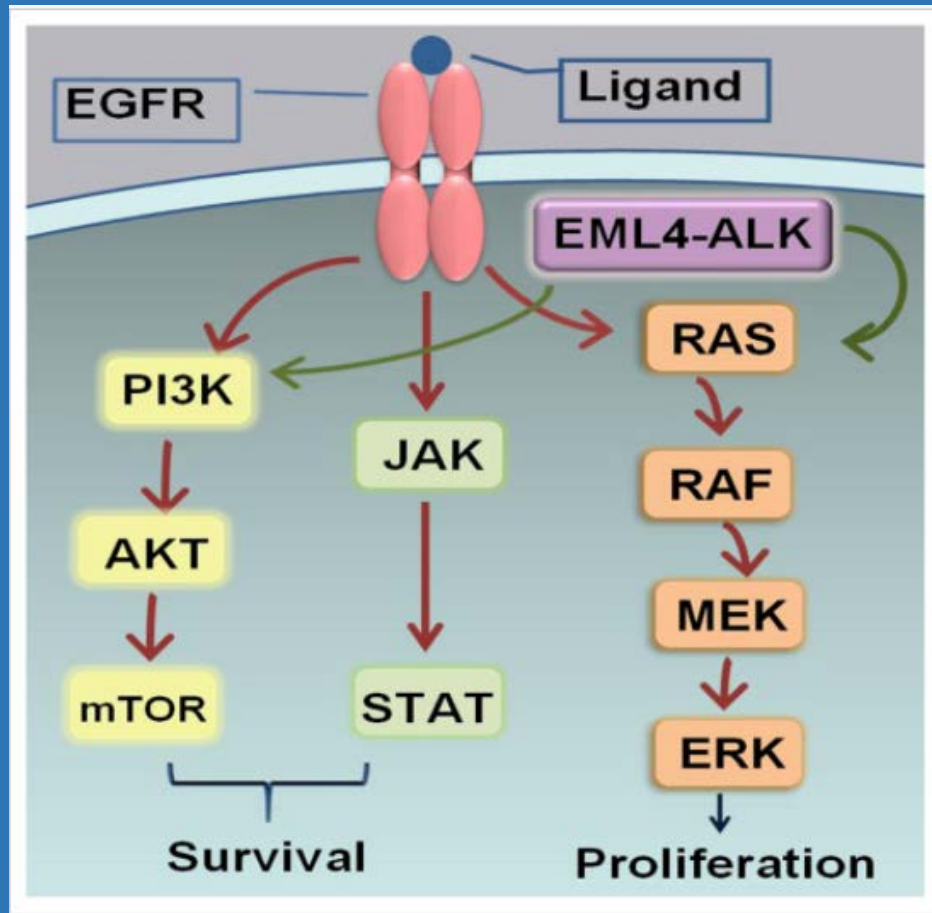
		Males	Females			
Lung & bronchus	84,590	27%		Lung & bronchus	71,280	25%
Colon & rectum	27,150	9%		Breast	40,610	14%
Prostate	26,730	8%		Colon & rectum	23,110	8%
Pancreas	22,300	7%		Pancreas	20,790	7%
Liver & intrahepatic bile duct	19,610	6%		Ovary	14,080	5%
Leukemia	14,300	4%		Uterine corpus	10,920	4%
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%

The Biology of Lung Cancer

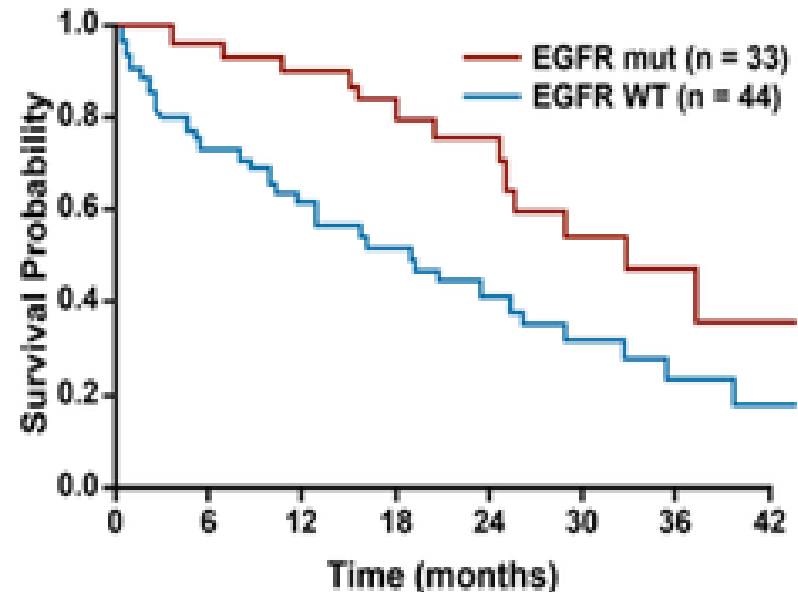
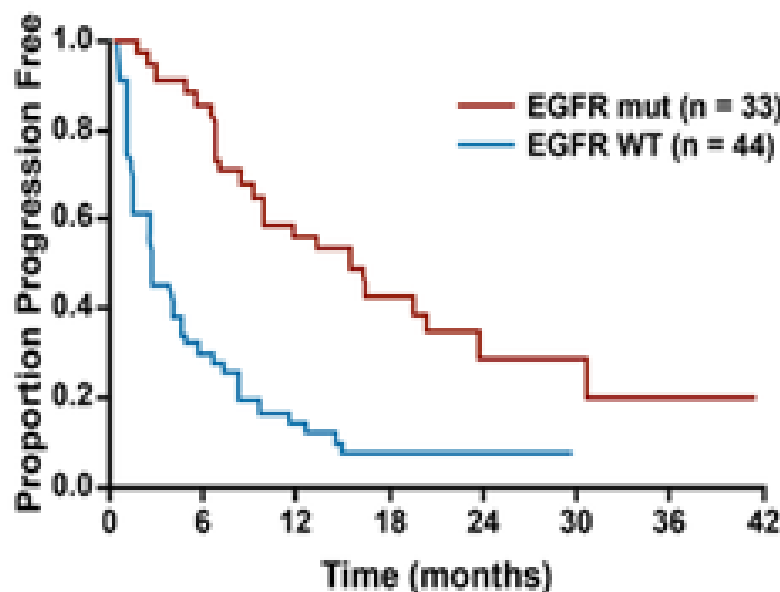


- Understanding histology and molecular landscape of NSCLC provides basis for treatment decisions.

Rationale for Targeting Specific Driving Mutations



EGFR TKIs Show More Benefit in EGFR Mutant Patients



RR

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

Mut = mutant; WT = wild type

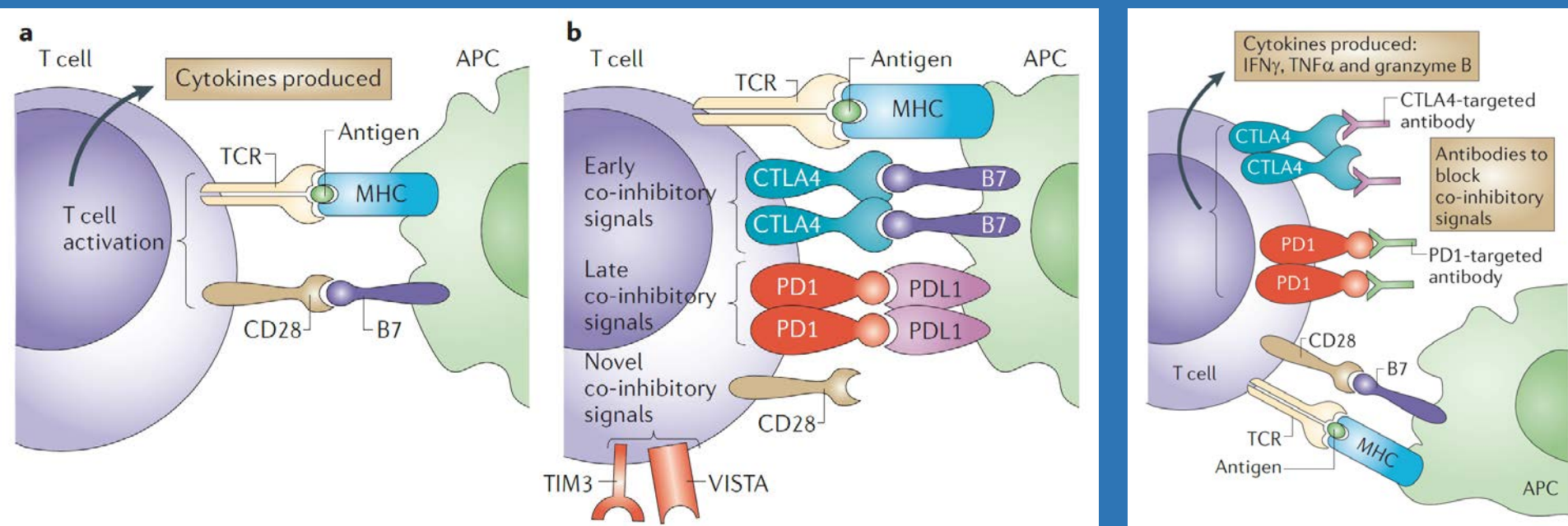
Janne PA, et al. *J Clin Oncol*. 2010;28:7503.



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.

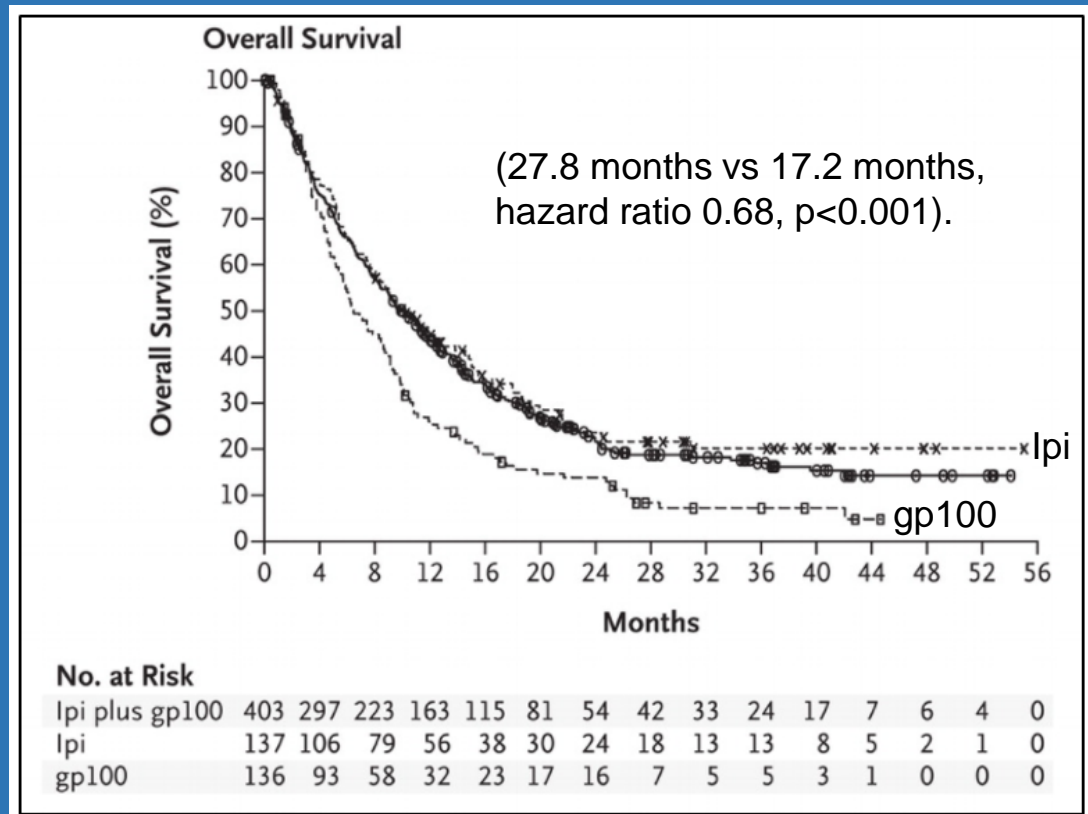
Immunotherapy Can Reverse Tumor Immune Escape



Sharma et al., NRC, 2011

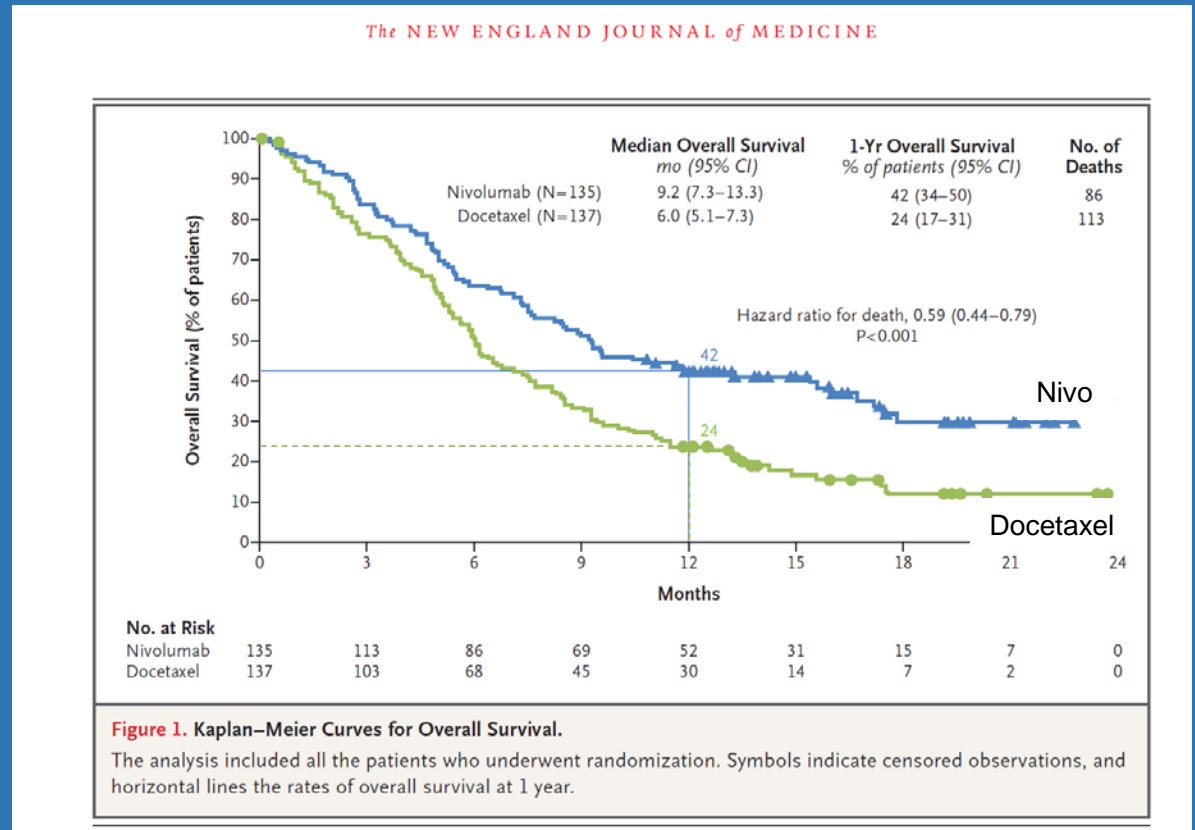
Blocking CTLA-4 is Therapeutically Efficacious in Melanoma

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



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.



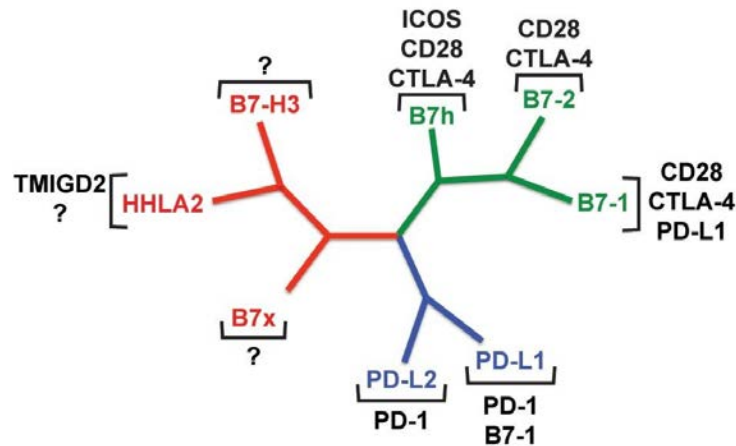
Brahmer et al., NEJM, 2015



Patients Treated with Both Specific TKIs and Immunotherapy Do Relapse

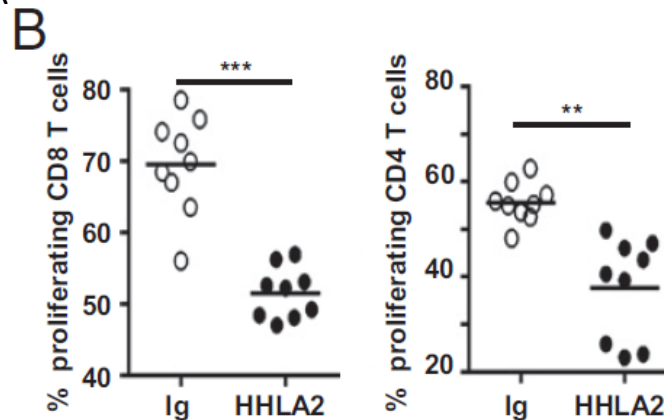
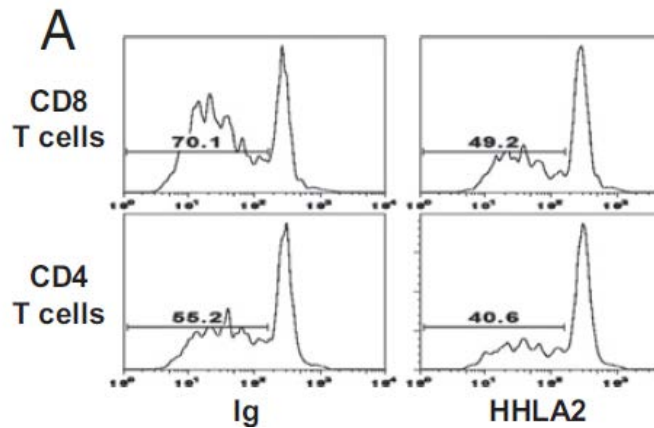
- There is an unmet need of alternative therapies for patients who relapse from initially beneficial drugs.
 - ❖ New Targets
 - ❖ Combination Therapies

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



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.

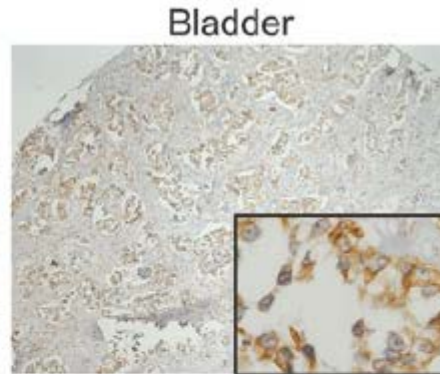
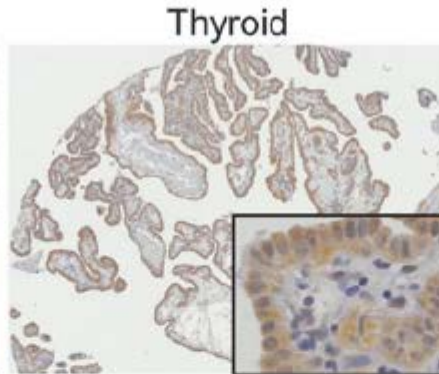
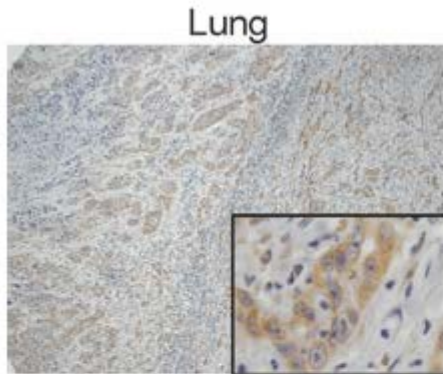
Janakarim et al., 2015, CCR



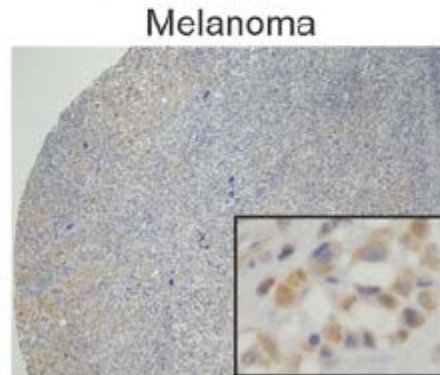
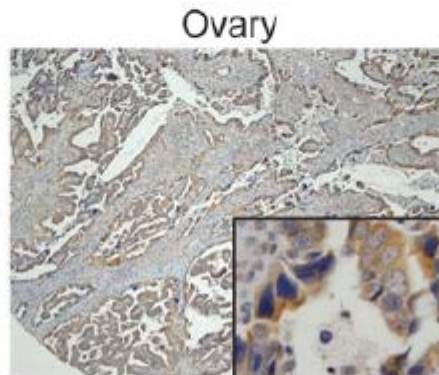
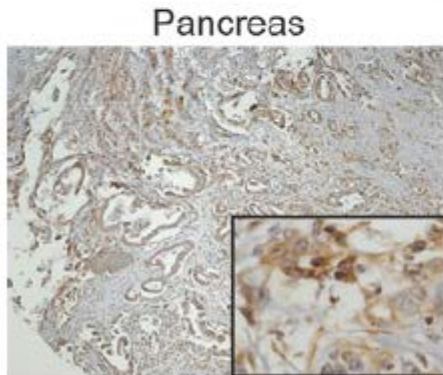
Zhao et al., 2013, PNAS

HHLA2 Is Expressed in Many Different Cancers

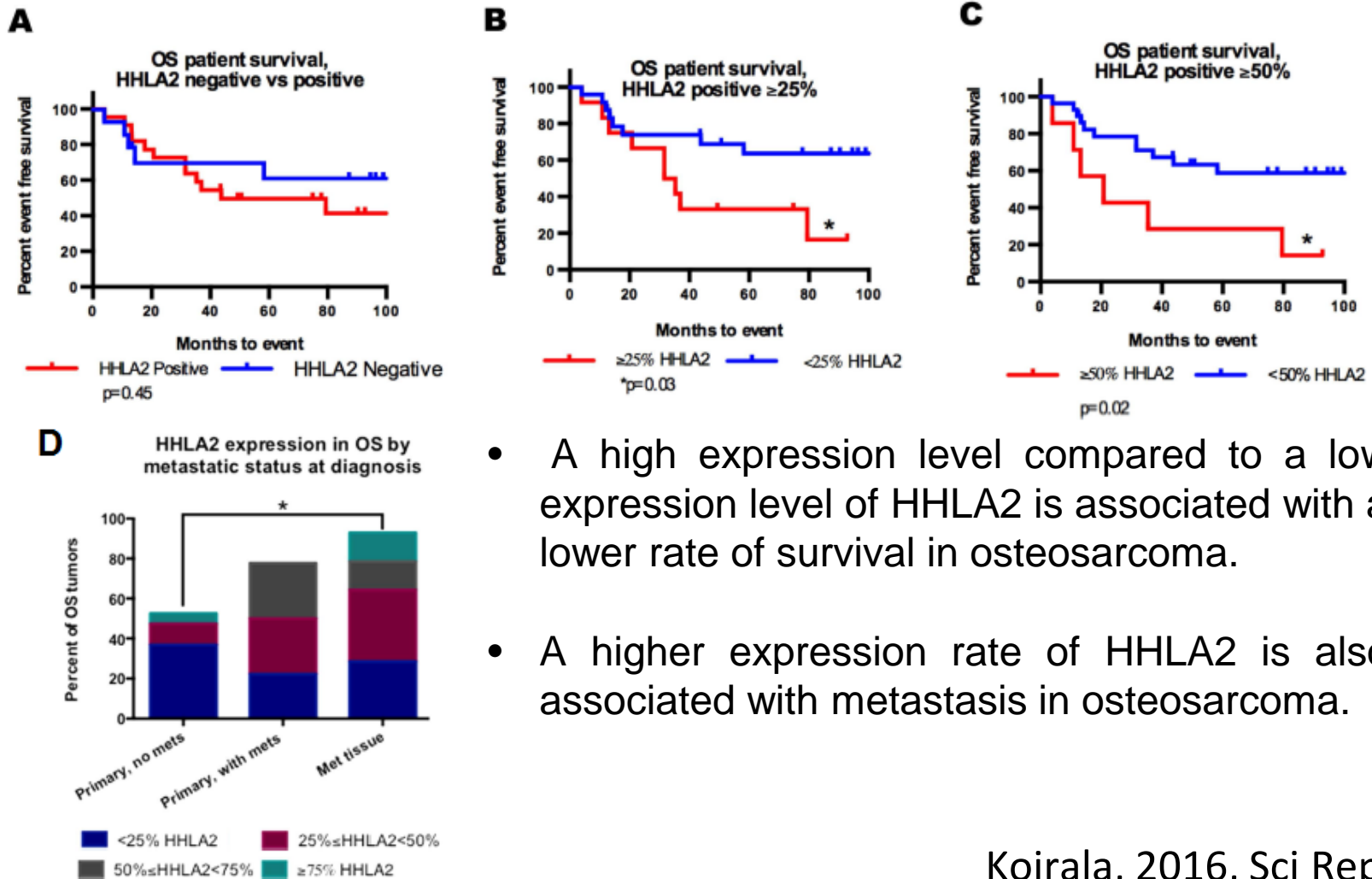
HHLA2



HHLA2



HHLA2 is Associated with Poor Survival and Metastasis in Osteosarcoma



HHLA2 is Widely Expressed in Lung Cancer

Parameter	Discovery cohort (n = 392)			P	Parameter	Validation cohort (n = 287)			
	HHLA2 Negative	HHLA2 Positive				HHLA2 Negative	HHLA2 Positive		
Age, year	67.9	67.5		0.78	Age, year	70.5	70		0.47
Gender				0.60	Gender				0.09
Female (n = 215)	78 (36%)	137 (64%)			Female (n = 180)	46 (26%)	134 (74%)		
Male (n = 141)	55 (39%)	86 (61%)			Male (n = 80)	29 (36%)	51 (64%)		
Histology				<0.0001	Histology				0.92
Adeno (n = 290)	91 (31%)	199 (69%)			Adeno (n = 186)	58 (31%)	128 (69%)		
Squam (n = 31)	20 (65%)	11 (35%)			Squam (n = 29)	8 (27%)	21 (73%)		
Large (n = 18)	16 (89%)	2 (11%)			Large (n = 3)	1 (33%)	2 (67%)		
Stage				0.09	Stage				0.39
I (n = 252)	85 (34%)	167 (66%)			I (n = 157)	43 (27%)	114 (73%)		
II (n = 47)	23 (49%)	24 (51%)			II (n = 39)	15 (38%)	24 (61%)		
III (n = 35)	10 (29%)	25 (71%)			III (n = 22)	7 (31%)	15 (69%)		
Mutation status				0.04	Mutation status				0.01
EGFR (n = 41)	10 (24%)	31 (76%)			EGFR (n = 44)	5 (11%)	39 (89%)		
KRAS (n = 62)	23 (37%)	39 (63%)			KRAS (n = 66)	24 (36%)	42 (64%)		
WT/WT (n = 91)	43 (47%)	48 (53%)			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:

HHLA2 Expression:
IHC and Flow Cytometry

Analysis of T-cell Subpopulations:
Flow Cytometry

Common T-Cell Markers

· CD45 · CD3 · CD4 or · CD8

Exhausted T-Cells

• PD-1
• TIM3
• LAG-3

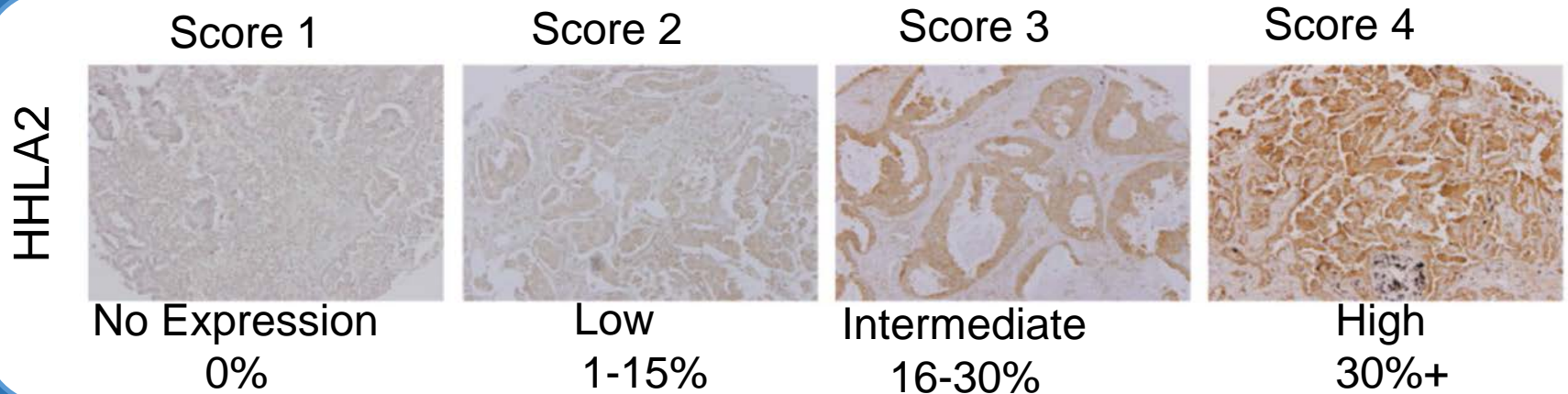
Proliferative and Active T-Cells

• Ki67
• Granzyme B
• CD38

Regulatory T-Cells

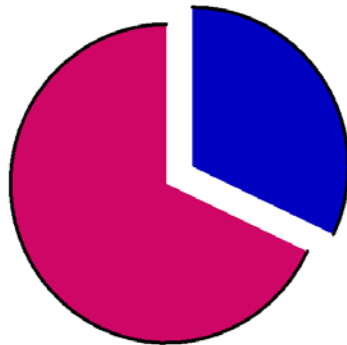
• CD25
• FoxP3

Aim 1.1: Expected Results



Acquired from Chiou et al., Scientific Reports, 2017

HHLA2 Expression:
Stage III and IV NSCLC



■ 32% Negative (16/50)
■ 68% Positive (34/50)

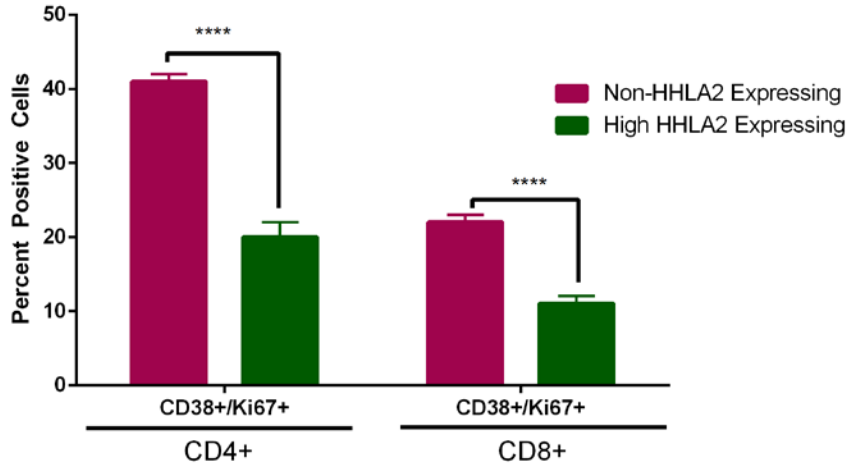
HHLA2 IHC Score:
Stage III and IV NSCLC



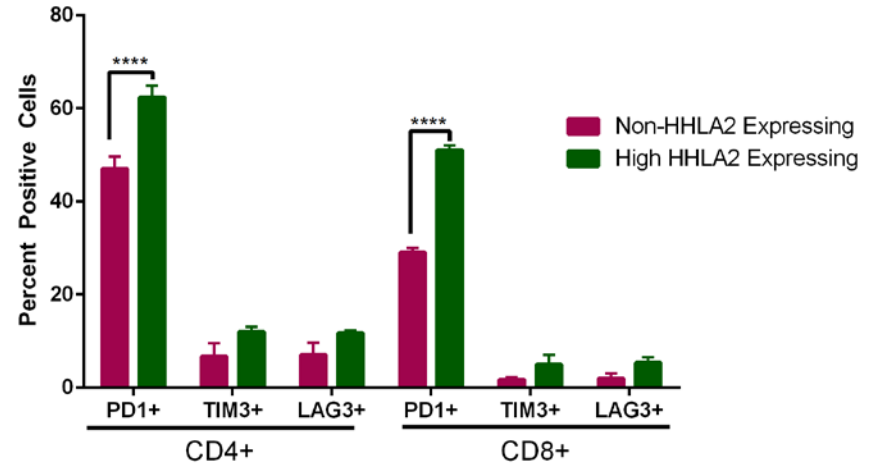
■ 32% Score 1 (16/50)
■ 6% Score 2 (3/50)
■ 26% Score 3 (13/50)
■ 36% Score 4 (18/50)

Aim 1.1 : Expected Results

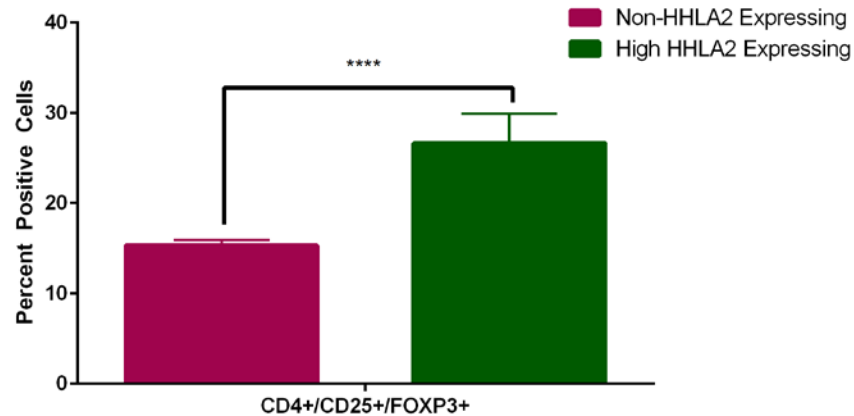
Percentage of Proliferative/Active T-Cells in Non- vs. High HHLA2 Expressing Tumors



Percentage of PD1, TIM3, or LAG3 Exhausted T-Cells in Non- vs. High HHLA2 Expressing Tumors

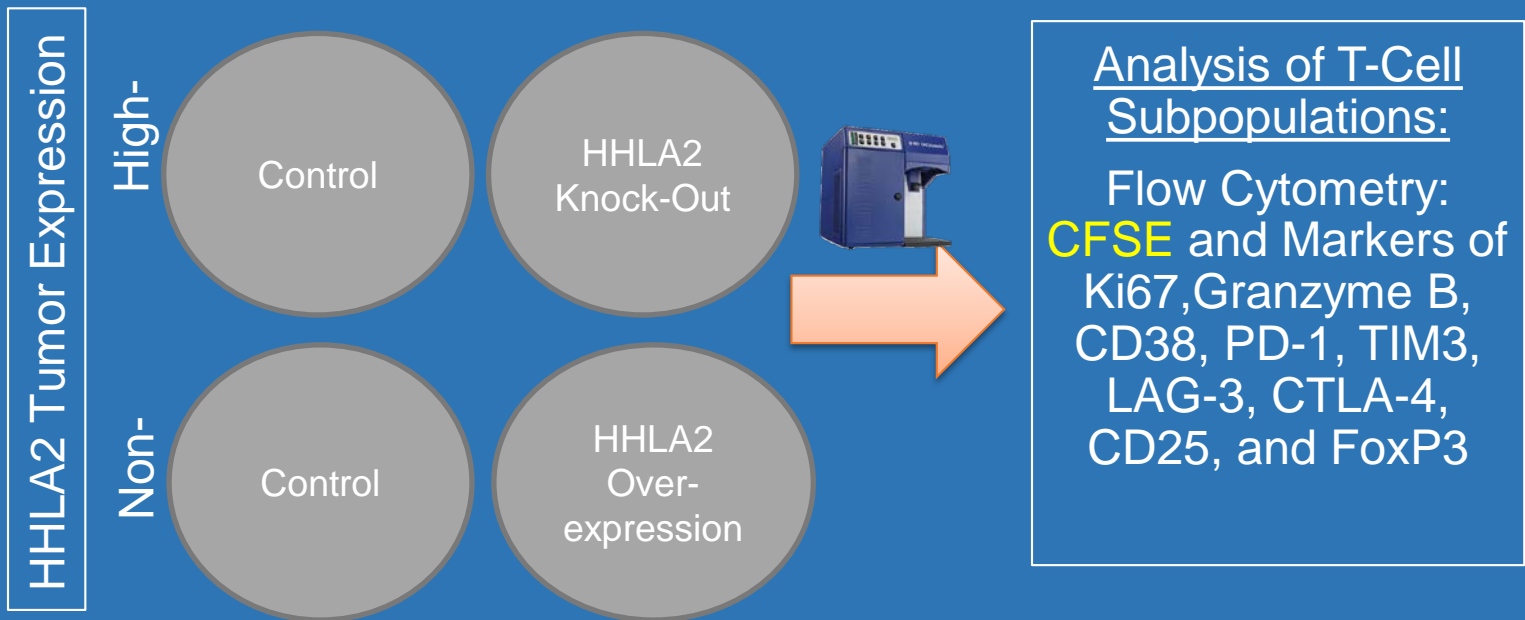


Regulatory T-Cells in Non- vs. High HHLA2 Expressing Tumors



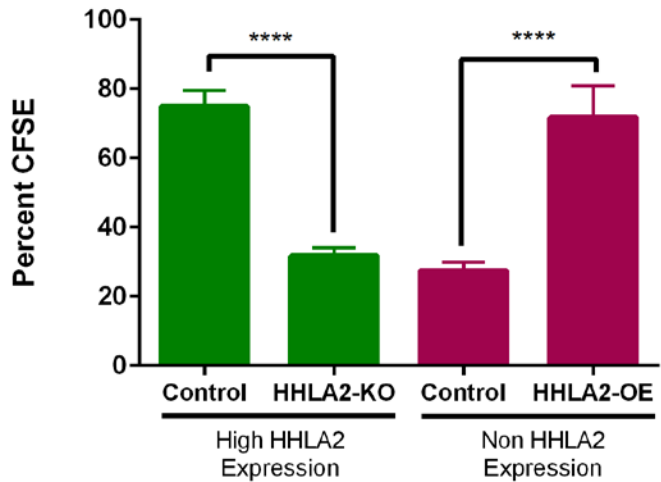
Aim 1.2: Examine the changes of T-cell subpopulations when HHLA2 is blocked within the TME.

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

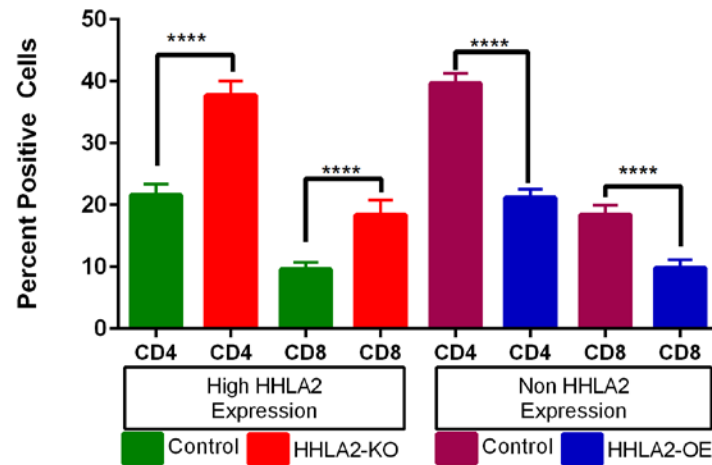


Aim 1.2: Expected Results

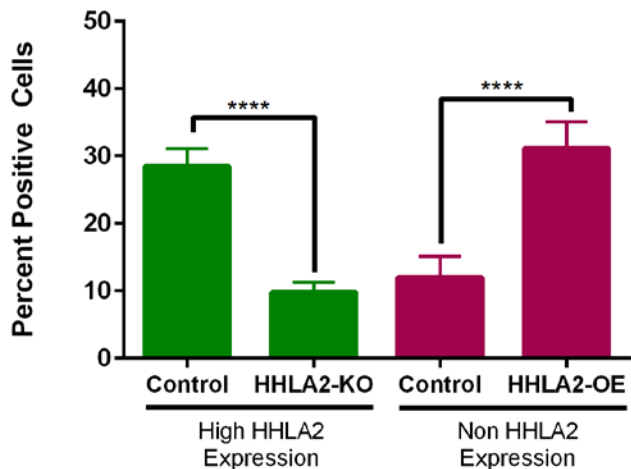
Percent of Non-Proliferating T-Cells



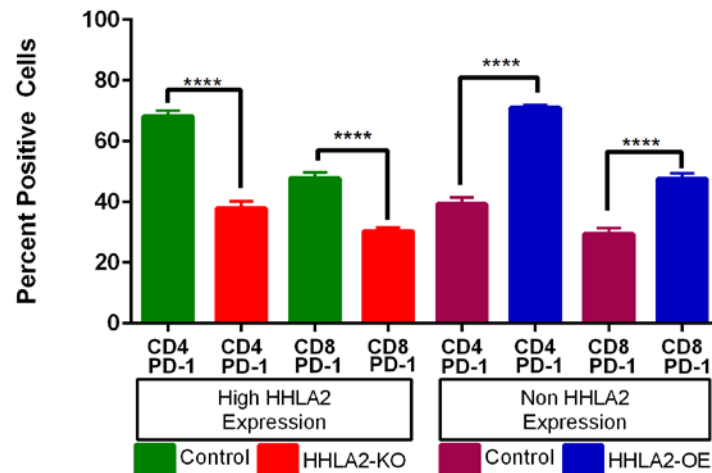
Percent of CD4+ or CD8+ Proliferative (CD38+/Ki67+/Granzyme B+) T-Cells



Percent of CD4+/CD25+/FOXP3+ Regulatory T-Cells



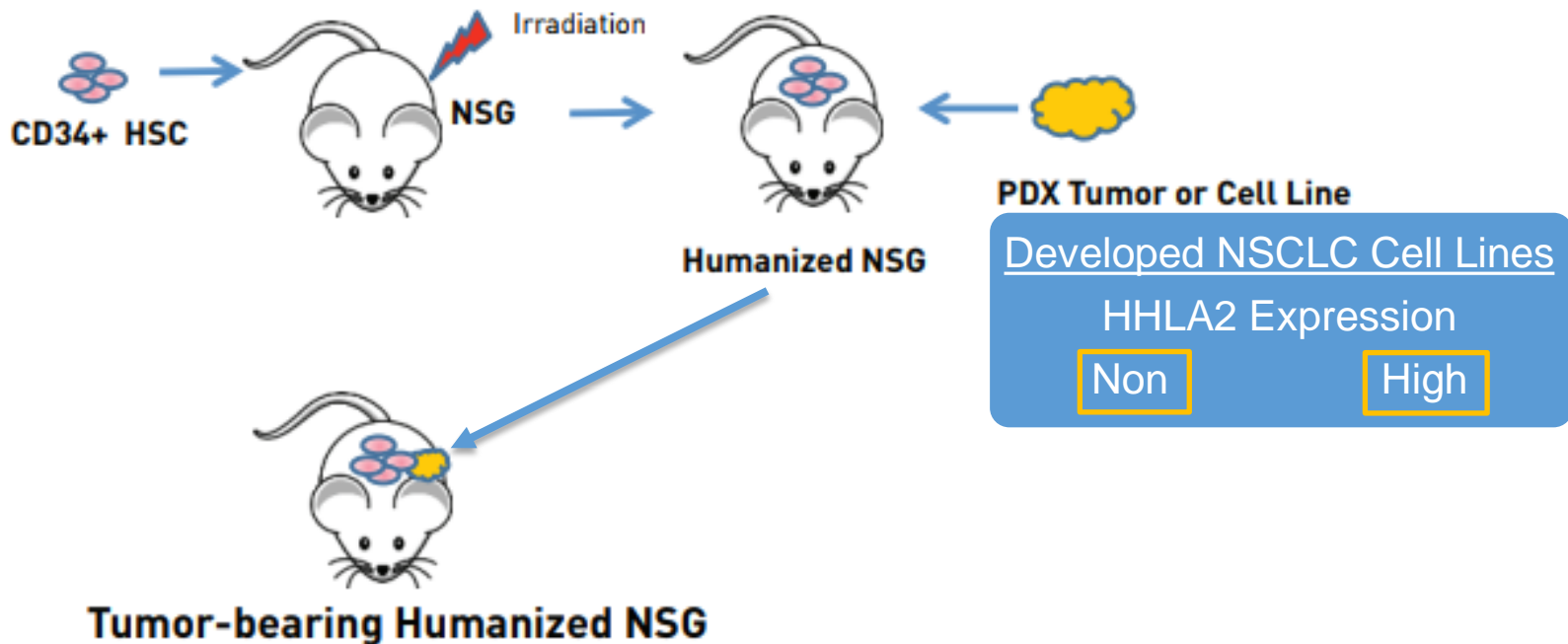
Percent of CD4+ or CD8+ Exhausted T-Cells



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

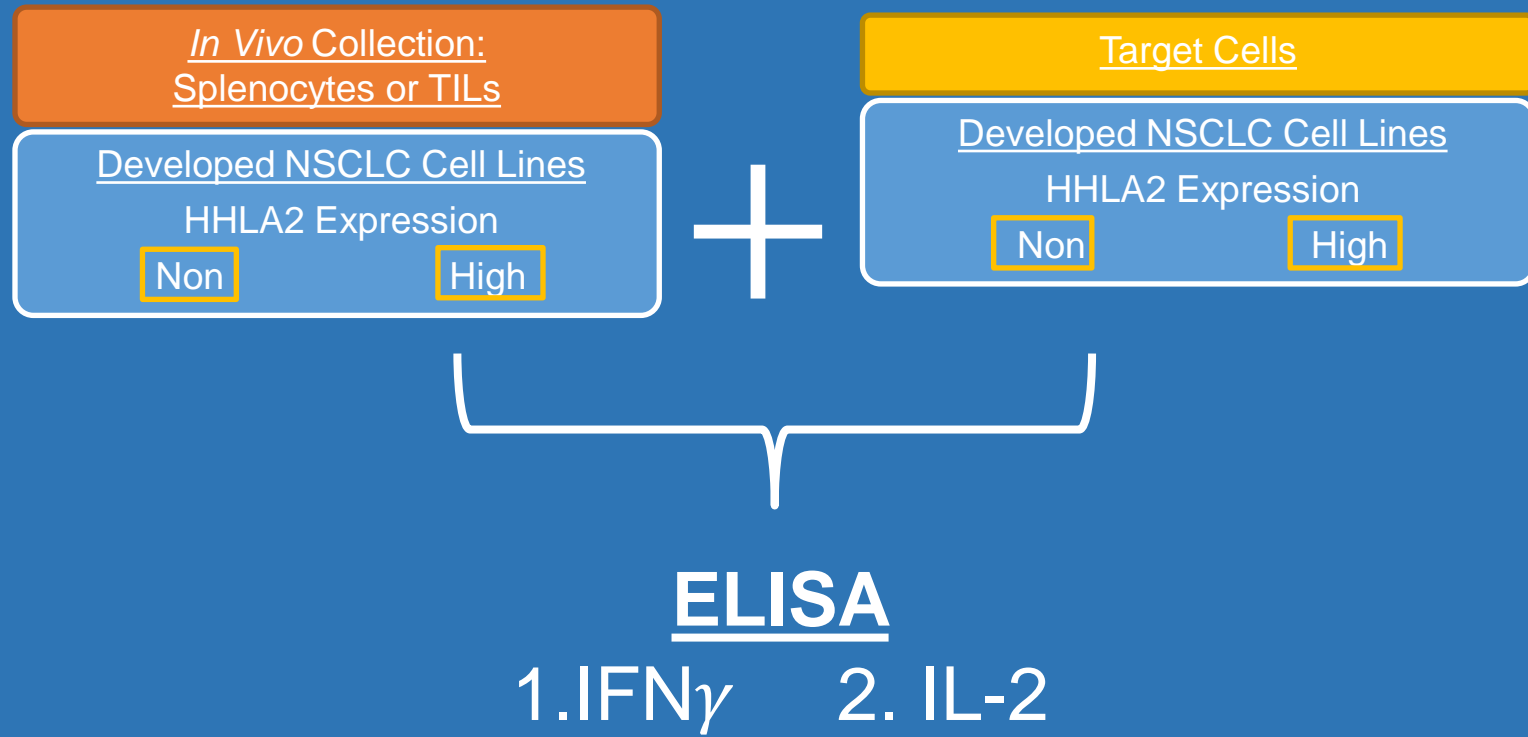
In Vivo Model

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



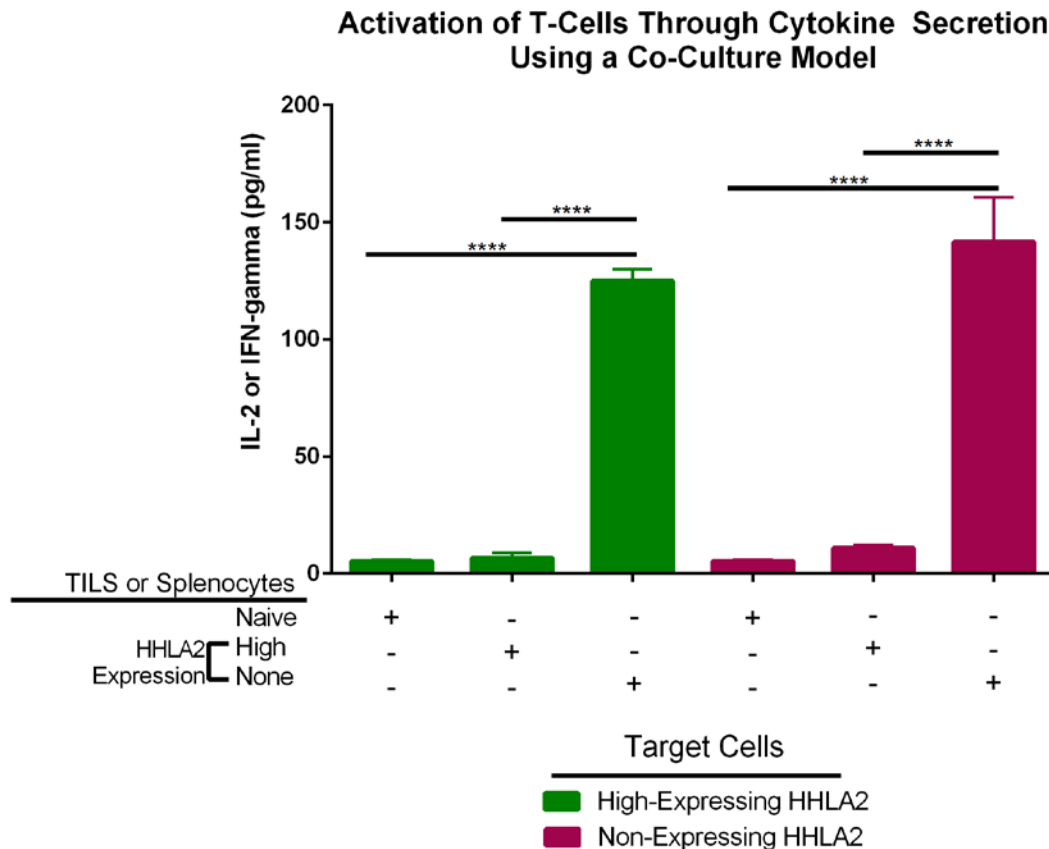
Aim 1.3: Investigate the activation of tumor infiltrating lymphocytes (TILs) or splenocytes of NSCLC bearing mice.

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



Aim 1.3: Expected Results

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



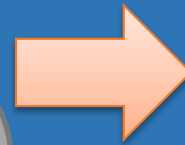
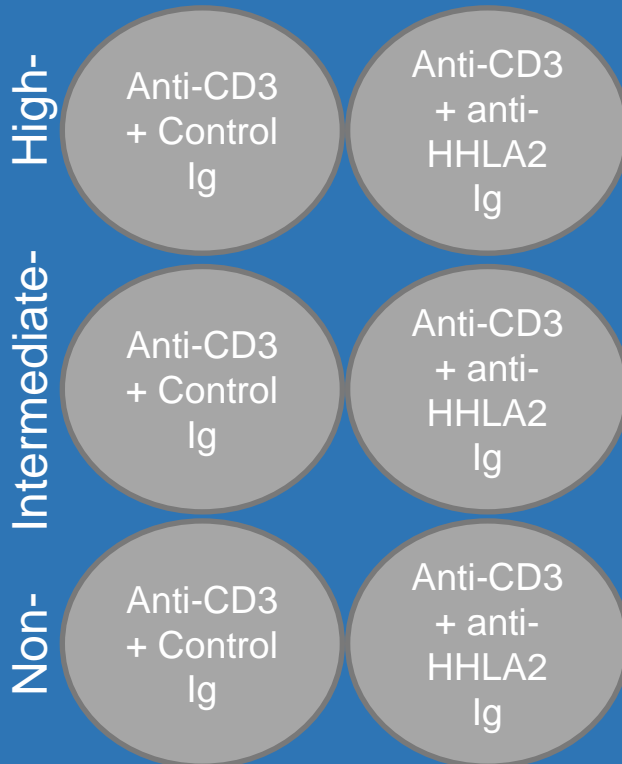
Aim 1: Potential Pitfalls and Alternatives

- 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.

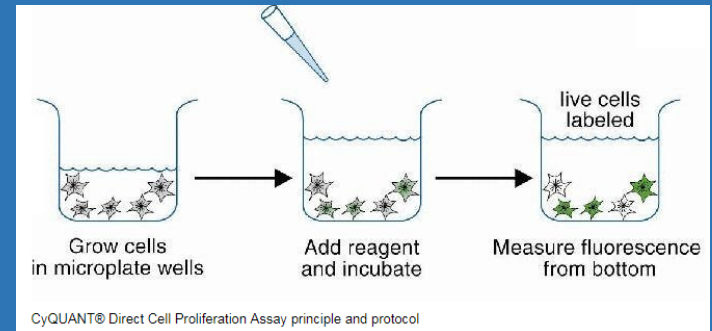
Aim 2.1: Test the therapeutic effect of a blocking HHLA2 in NSCLC.

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

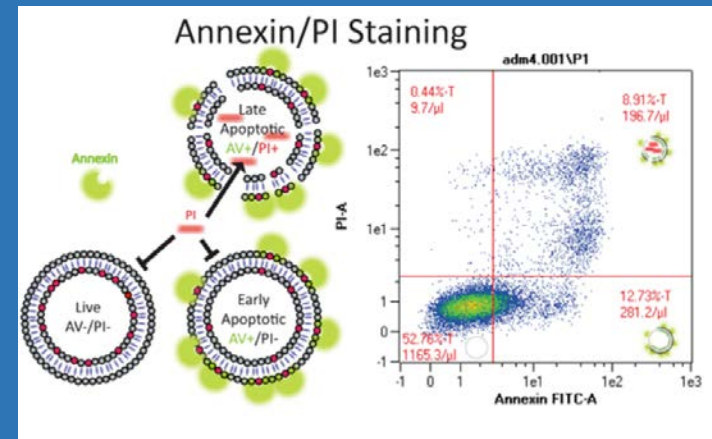
HHLA2 Tumor Expression



Measure Cell Growth: CyQUANT Assay

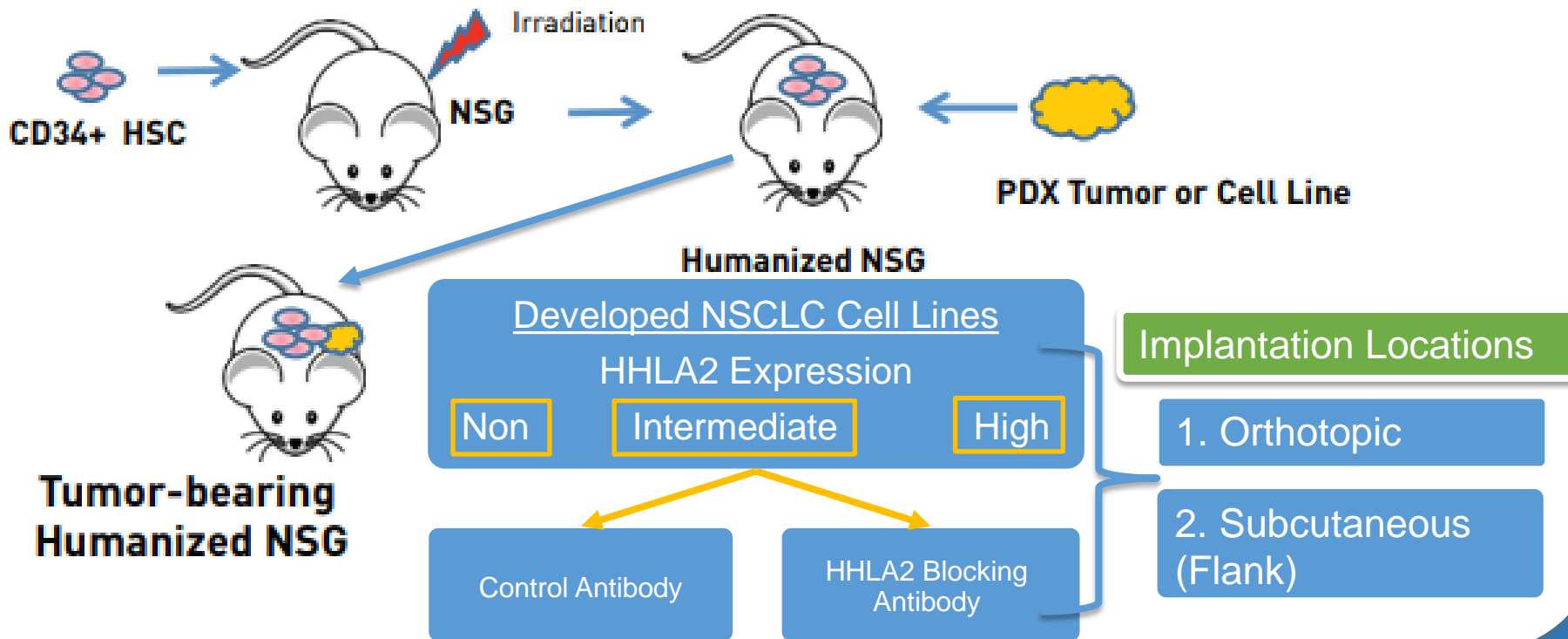


Apoptosis: Annexin Assay



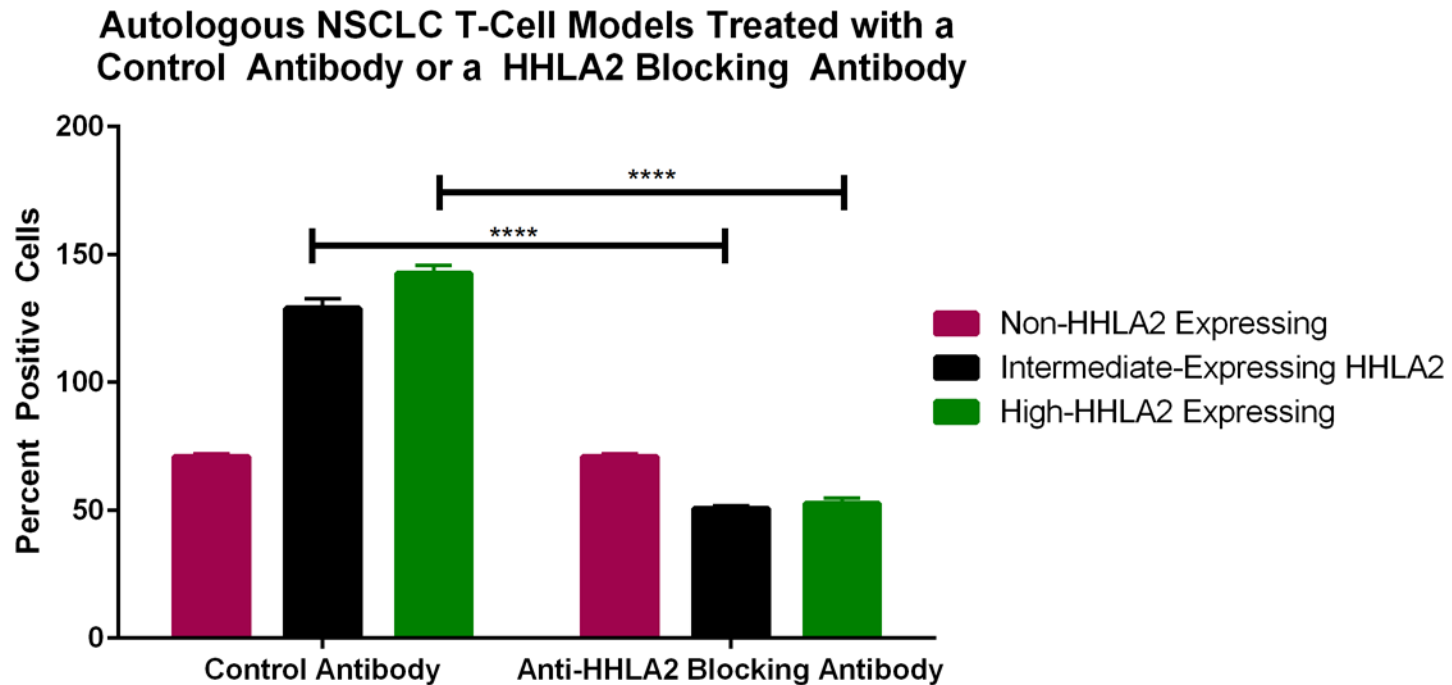
In Vivo Model

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



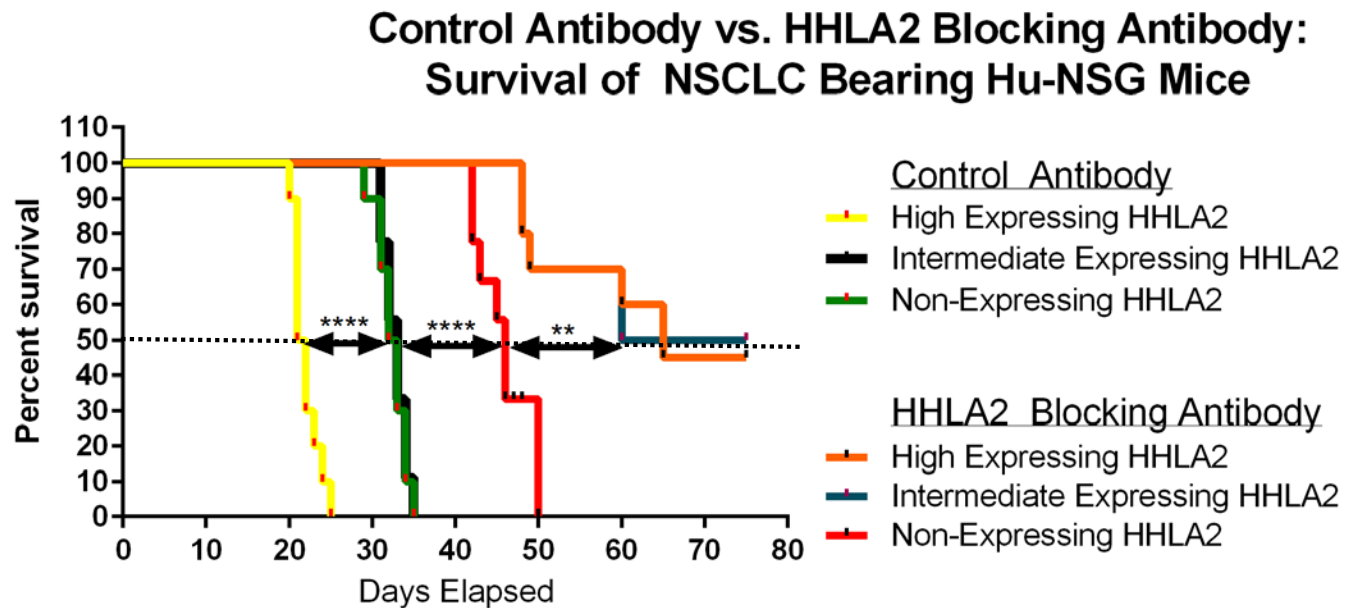
Aim 2.1: Expected Results

- 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.



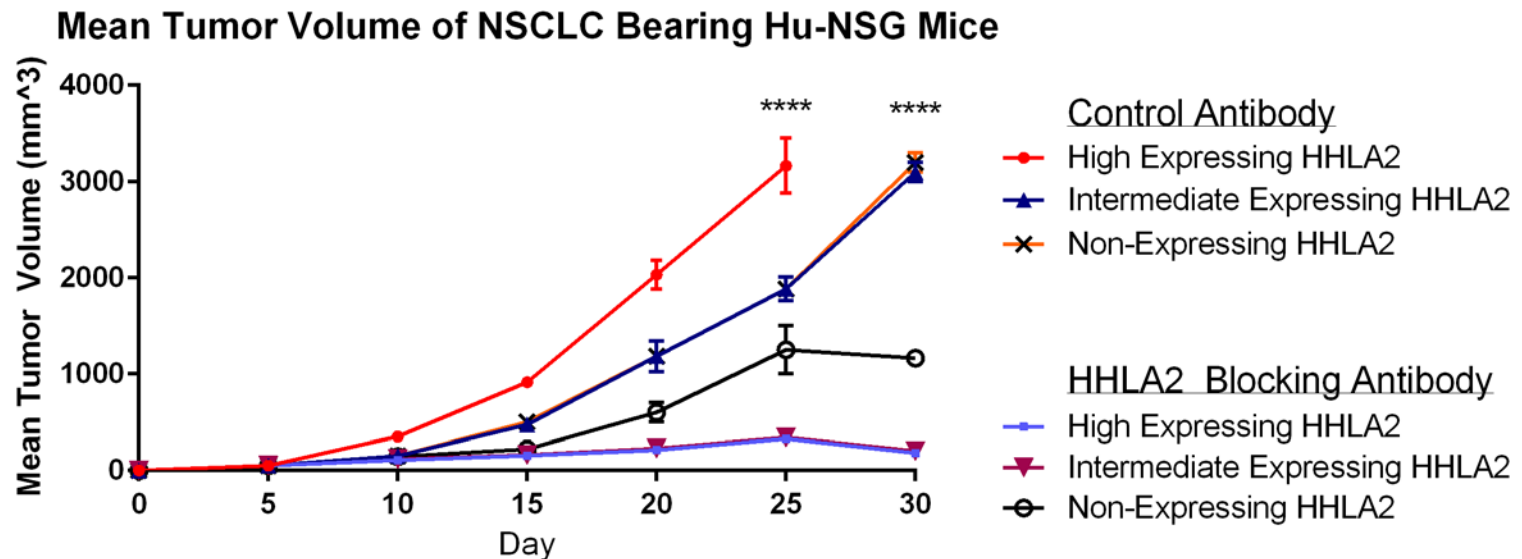
Aim 2.1: Expected Results

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



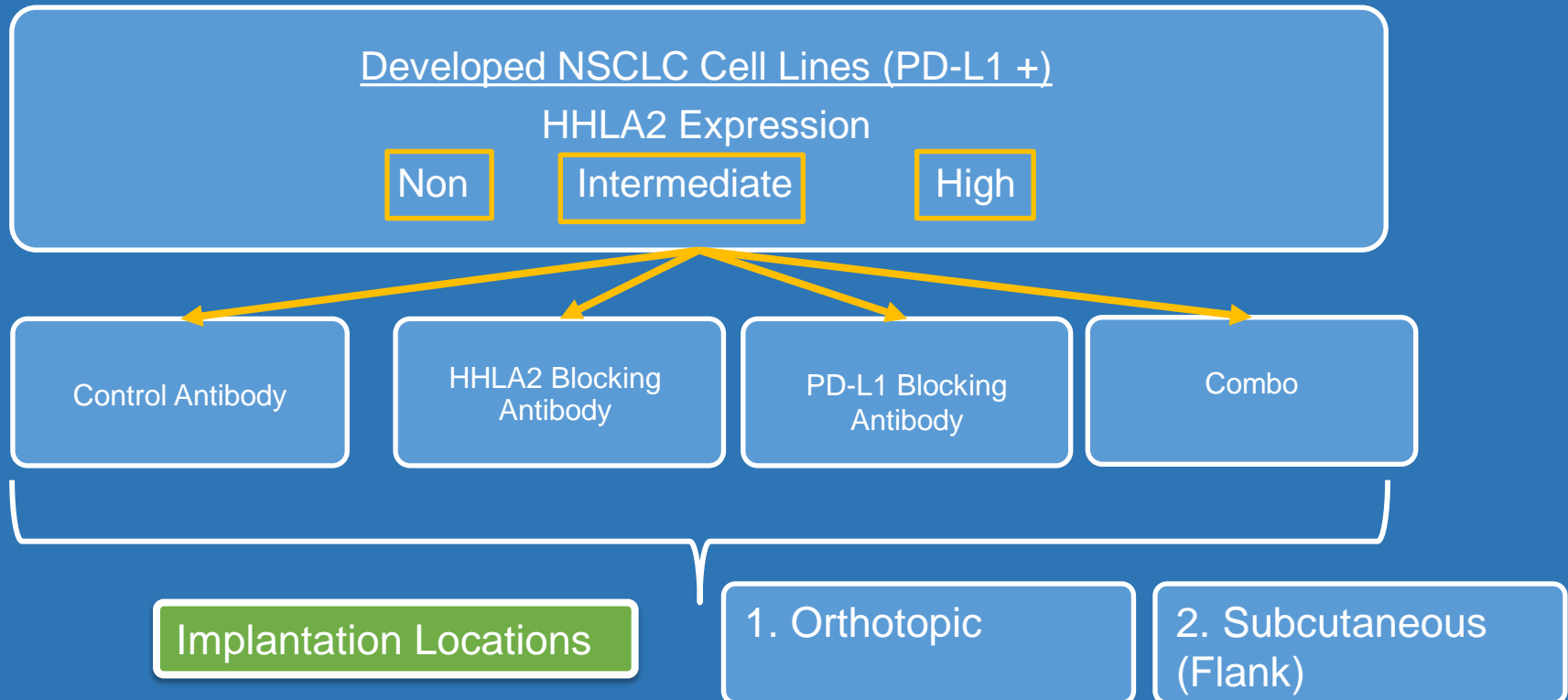
Aim 2.1: Expected Results

- 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*.

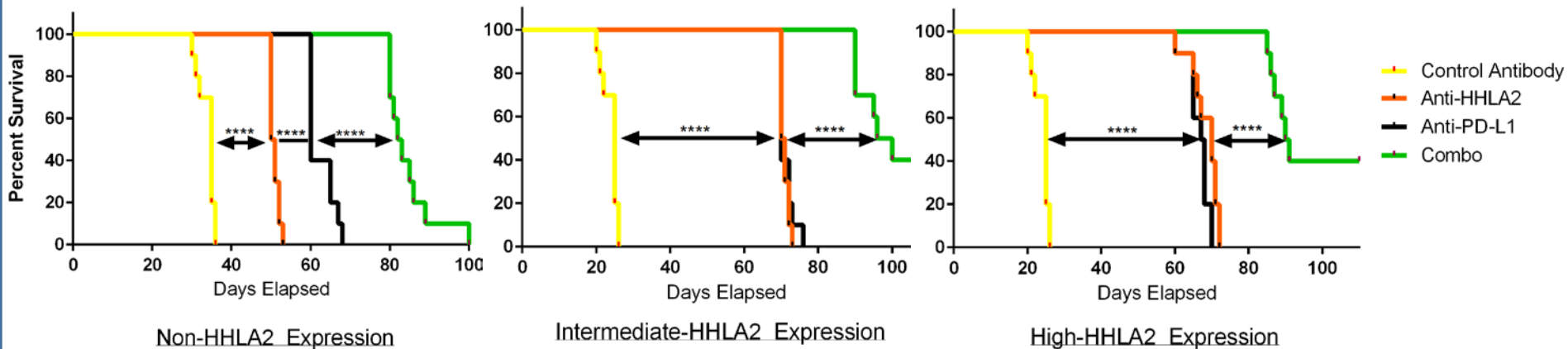
- Model: Humanized NSG Mouse Model
- Methods: Survival Experiments and Tumor Measurements



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.

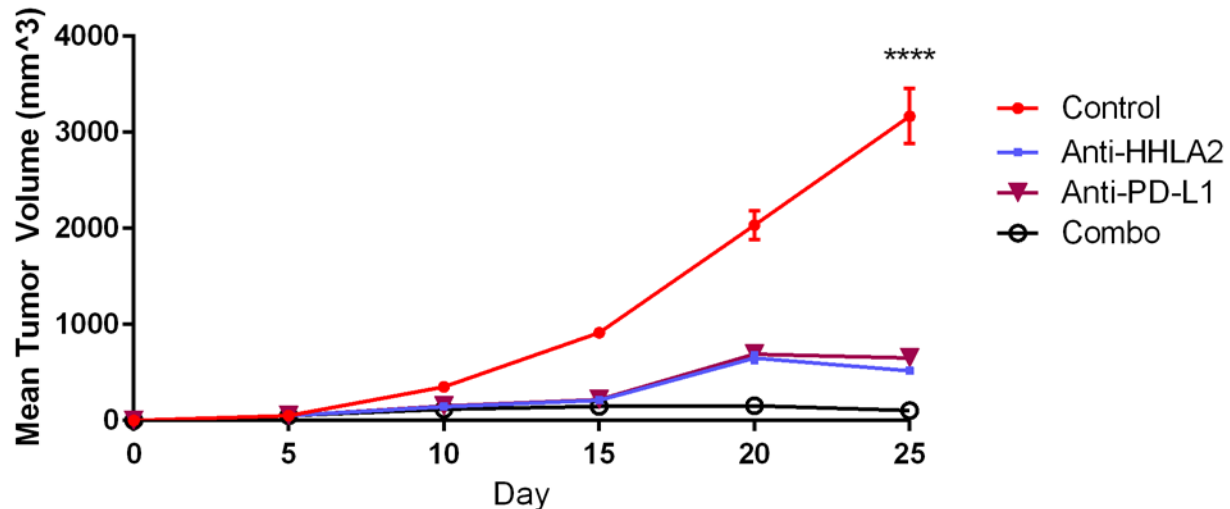
Survival of NSCLC Bearing Hu-NSG Mice



Aim 2.2: Expected Results

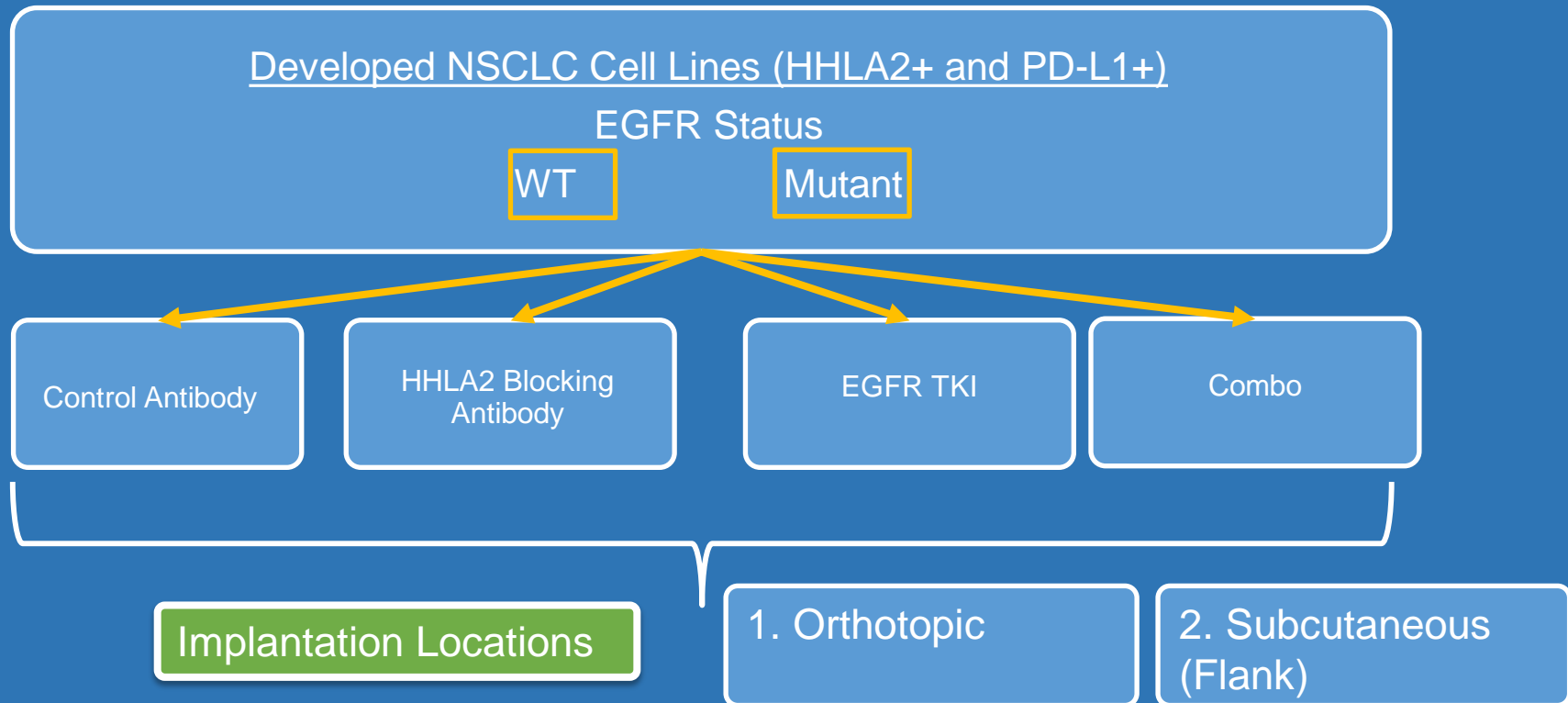
- I hypothesize that targeting both HHLA2 and PD-L1 in an HHLA2-overexpressing NSCLC model will delay growth of the tumor compared to other treated groups.

Mean Tumor Volume of PD-L1+/HHLA2+ NSCLC Bearing Hu-NSG Mice



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

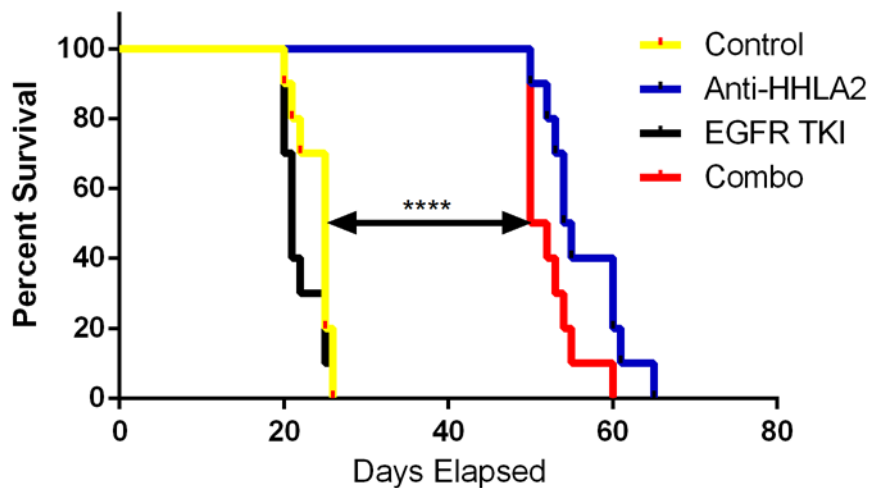
- Model: Humanized Lung Cancer Mouse Model
- Methods: Survival Experiments and Tumor Measurements



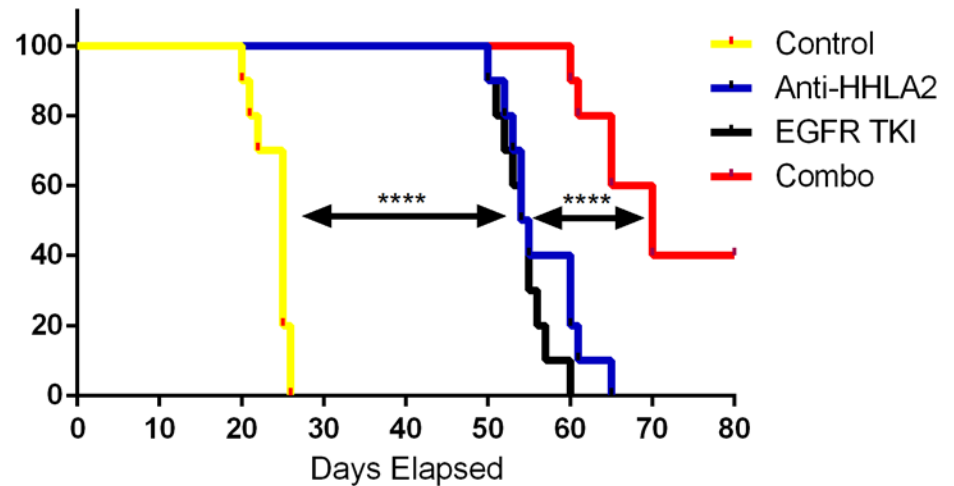
Aim 2.3: Expected Results

- 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.

Survival of NSCLC Bearing Hu-NSG Mice

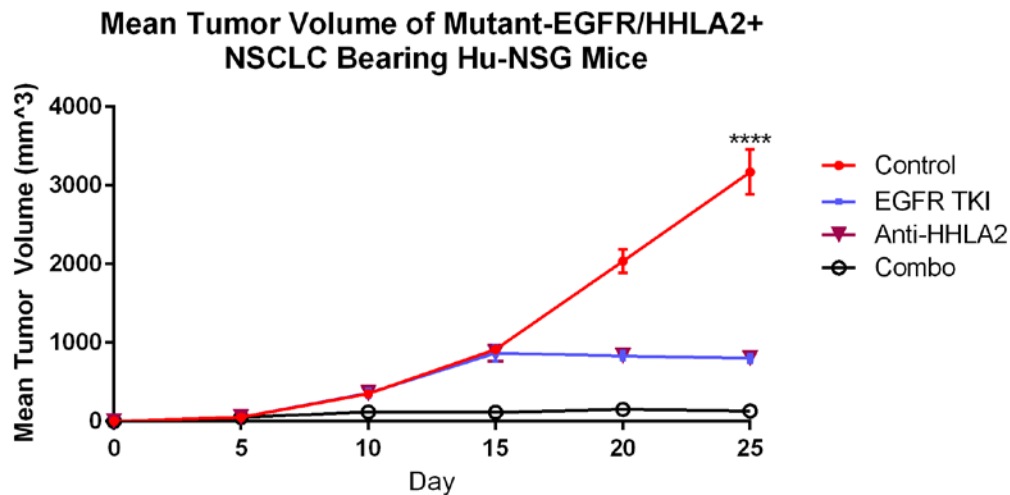
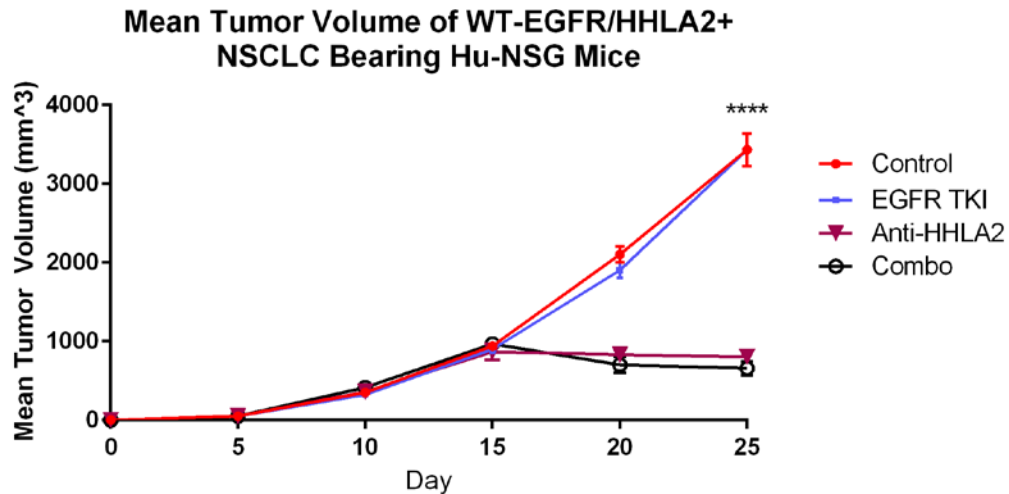


EGFR WT



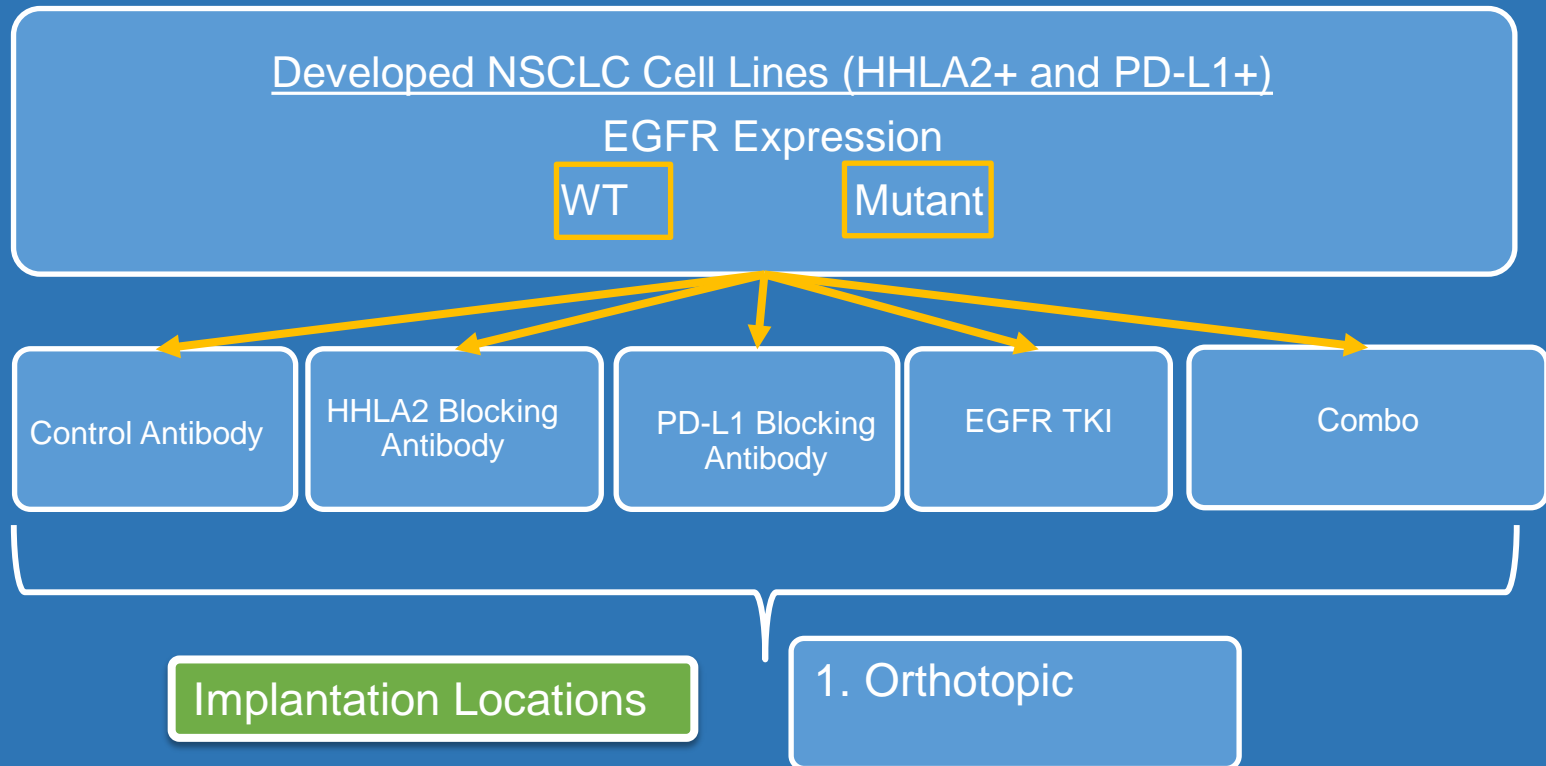
EGFR Mutant

Aim 2.3: Expected Results



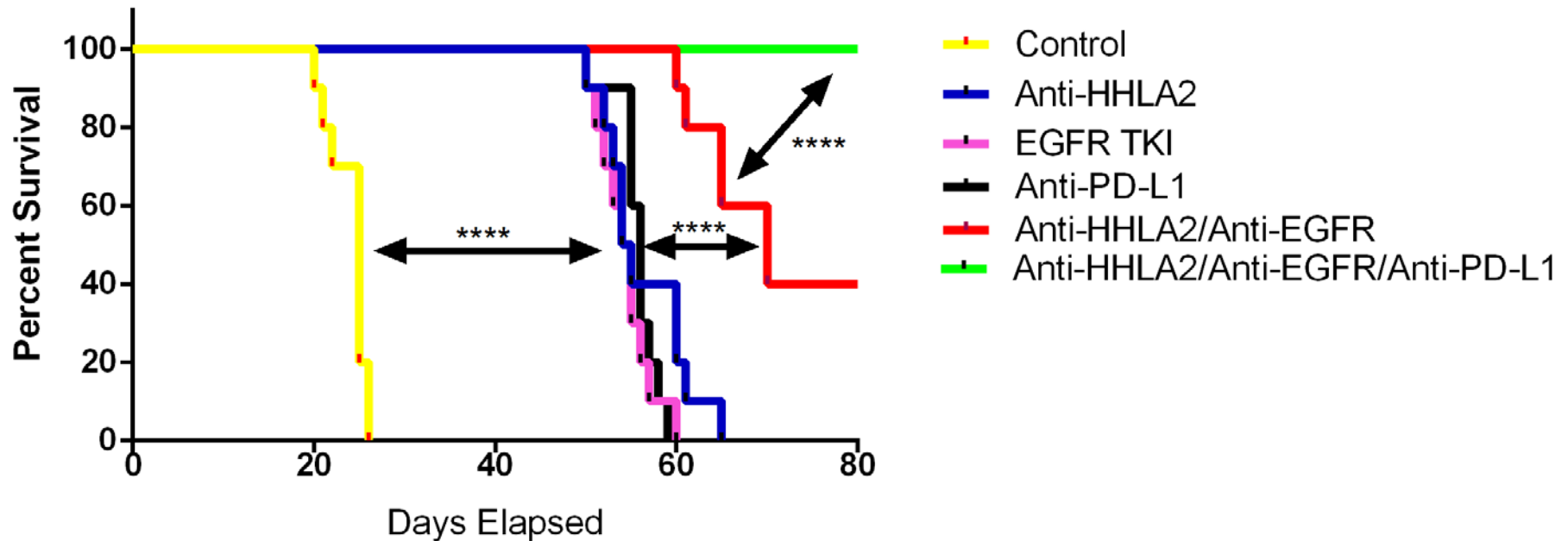
Aim 2.3 Extension: Inspect therapeutic effect of blocking EGFR, PD-L1, and HHLA2 in EGFR mutant NSCLC *in vivo*.

- 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.

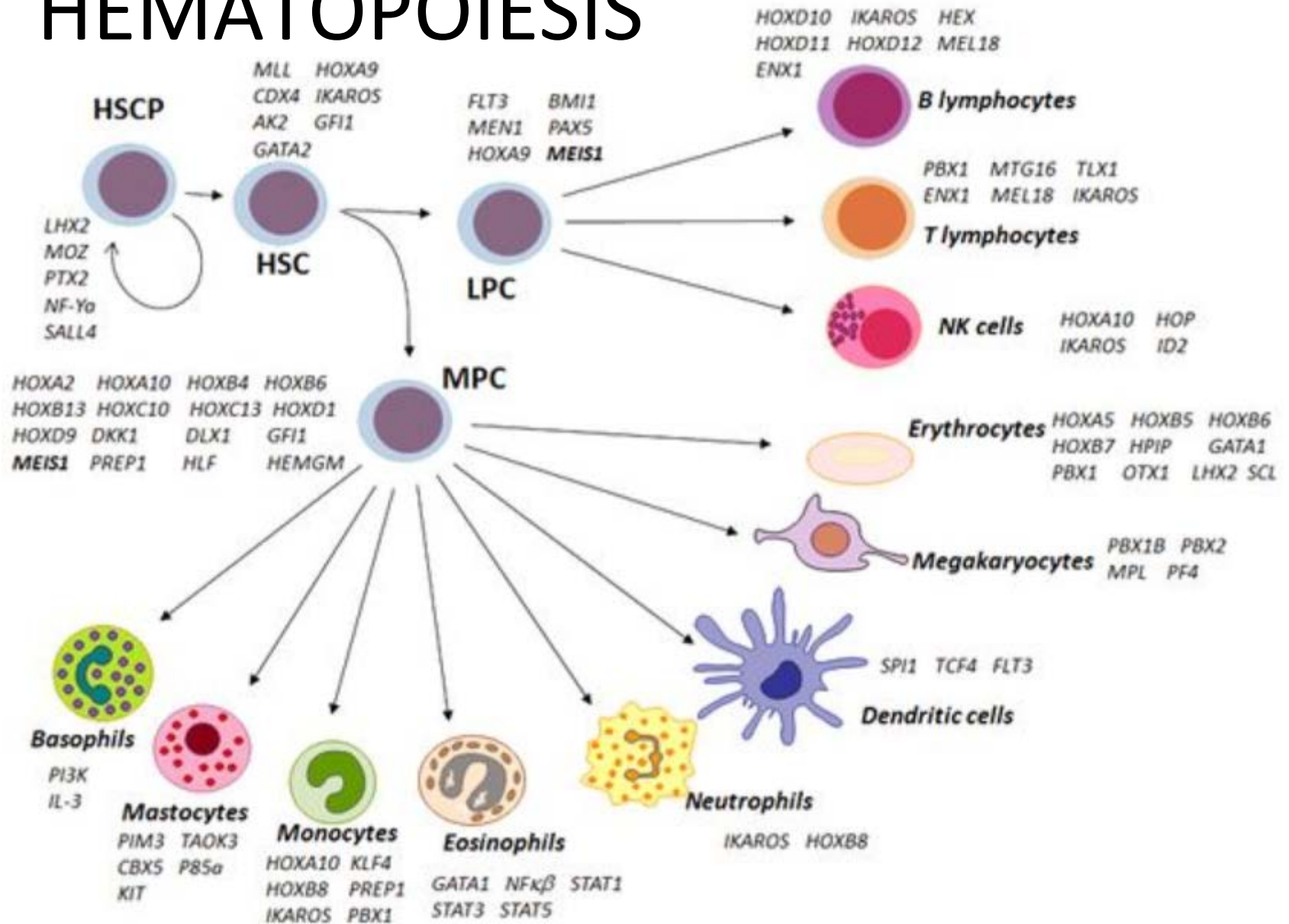
Conclusions

- 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!

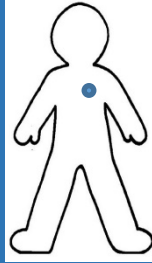


HEMATOPOIESIS

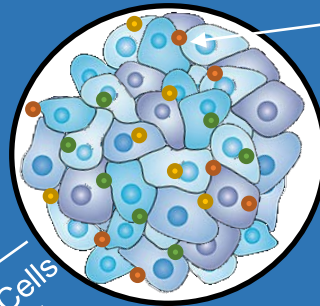


Development of lung cancer cell lines and matched T-cells

Lung Cancer Patient



Biopsy



TIL

Culture Cancer Cells in complete RPMI

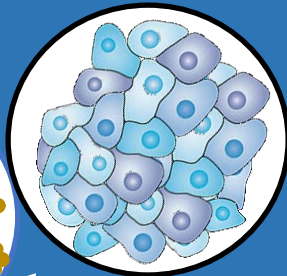
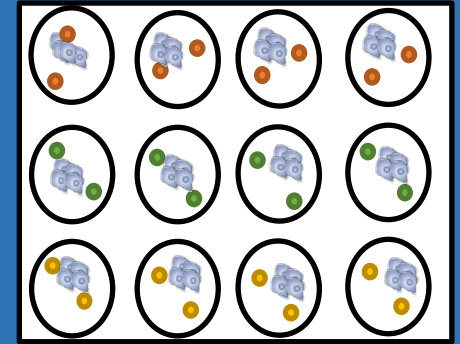
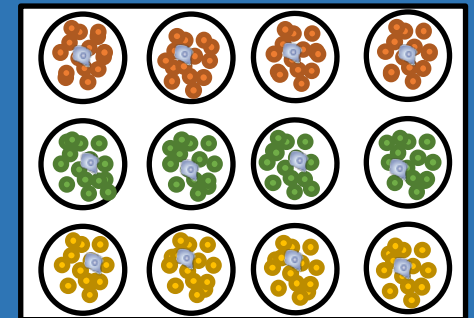


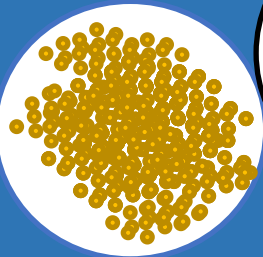
Plate Fragments



TIL Enrichment: Culture with IL-2



Expand + TILs



Common T-Cell Markers: CD45, CD3, CD8 or CD4

Proliferative and Active T-Cells

- Ki67
- Granzyme B
- CD38

Exhausted T-Cells

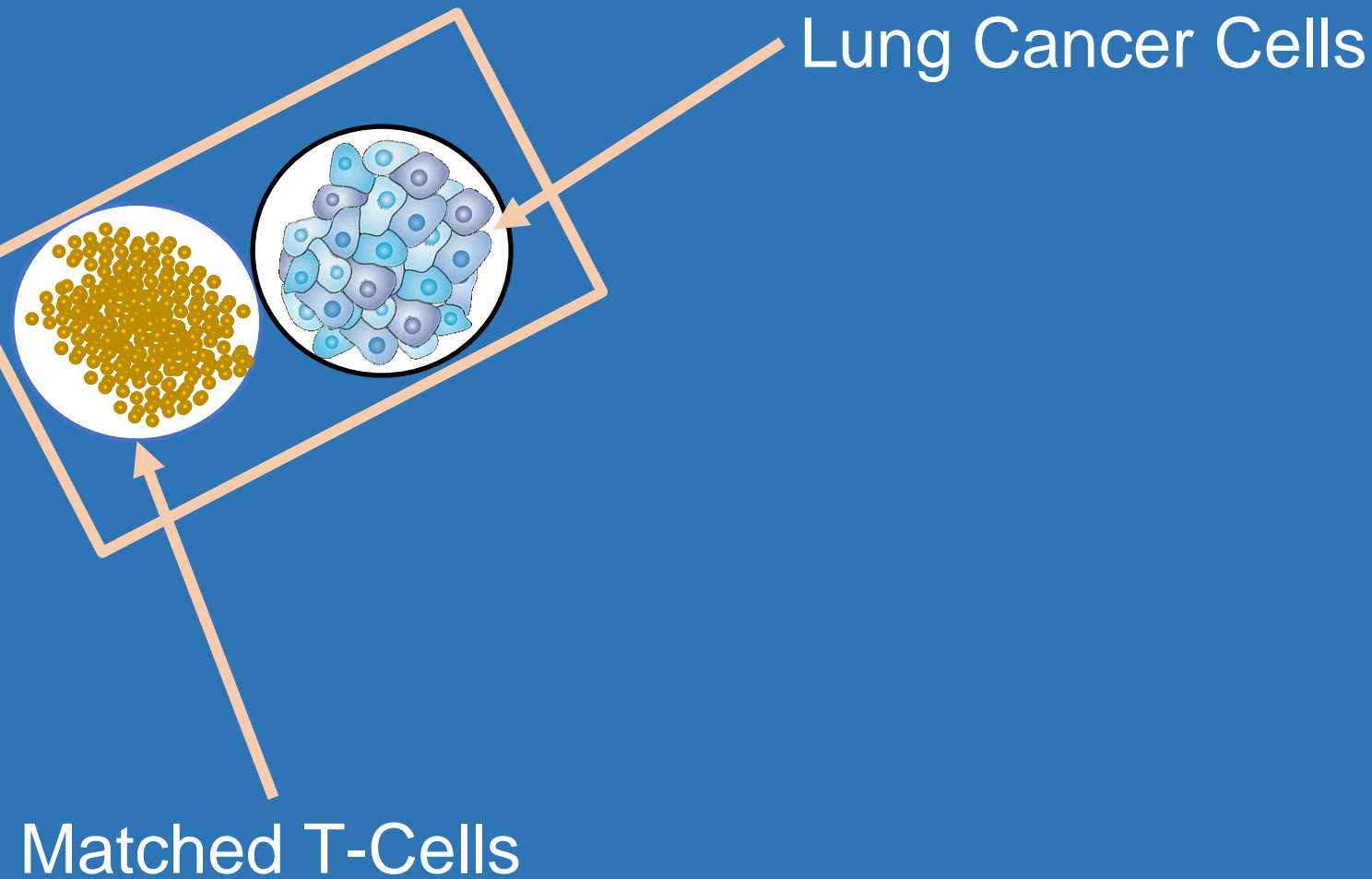
- PD-1
- TIM3
- LAG3

Regulatory T-Cells

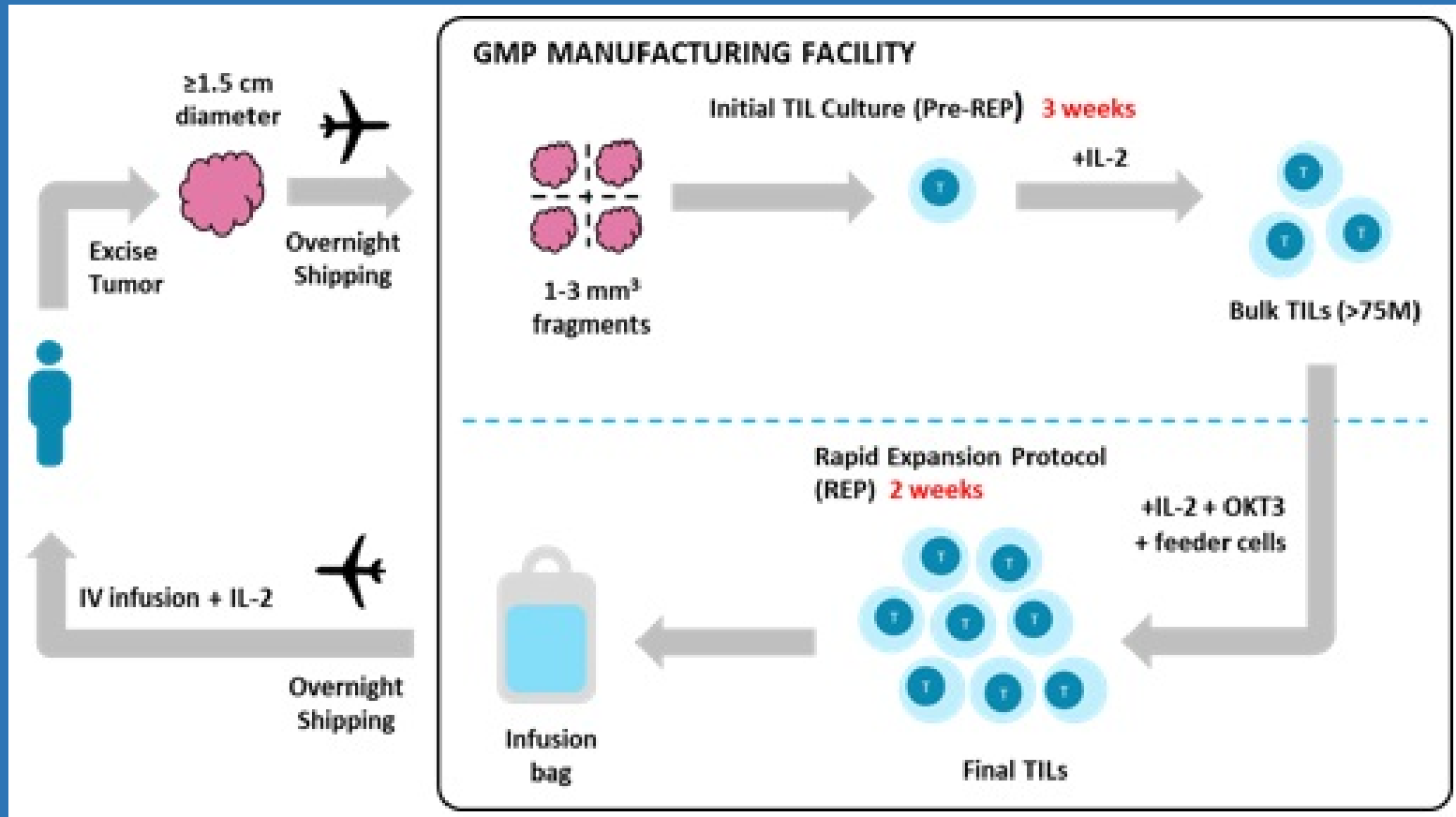
- CD25
- FoxP3

Characterize TILs by FACS Analysis

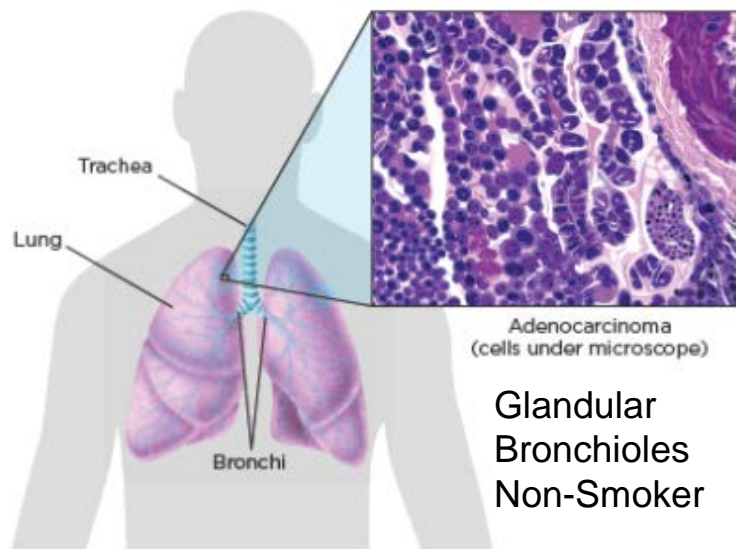
The Human Lung Cancer Model: Cancer Cells with Matched T-Cells



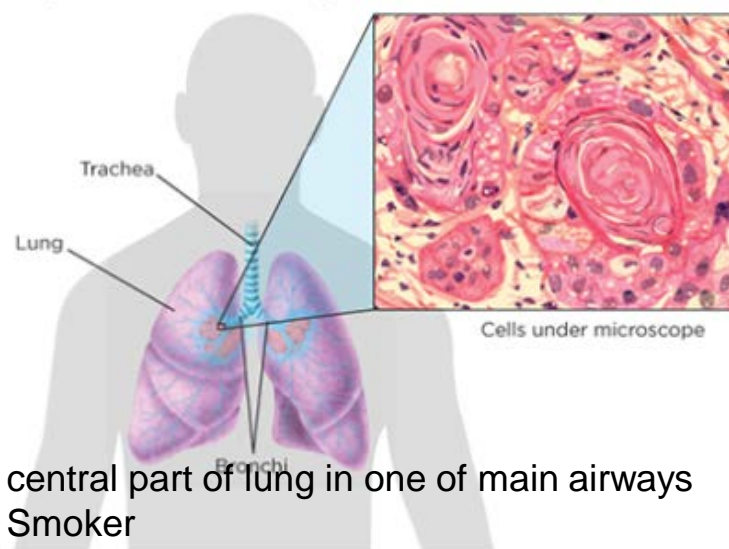
Rapid Expansion



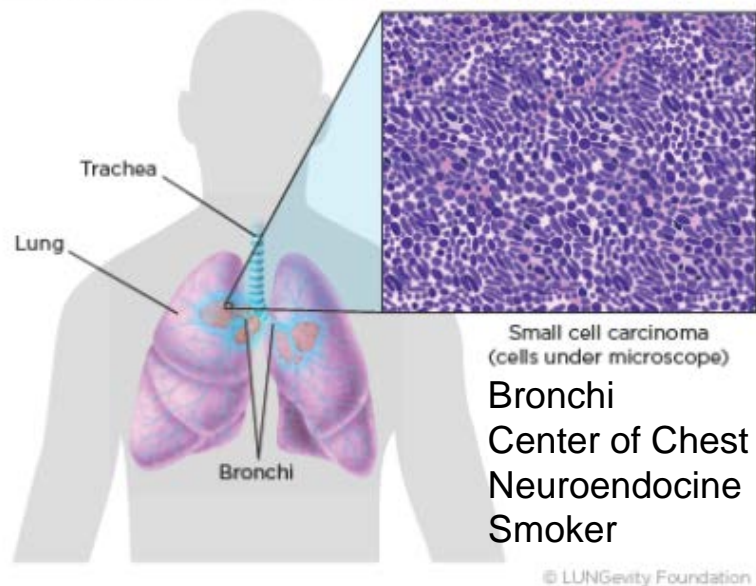
Adenocarcinoma

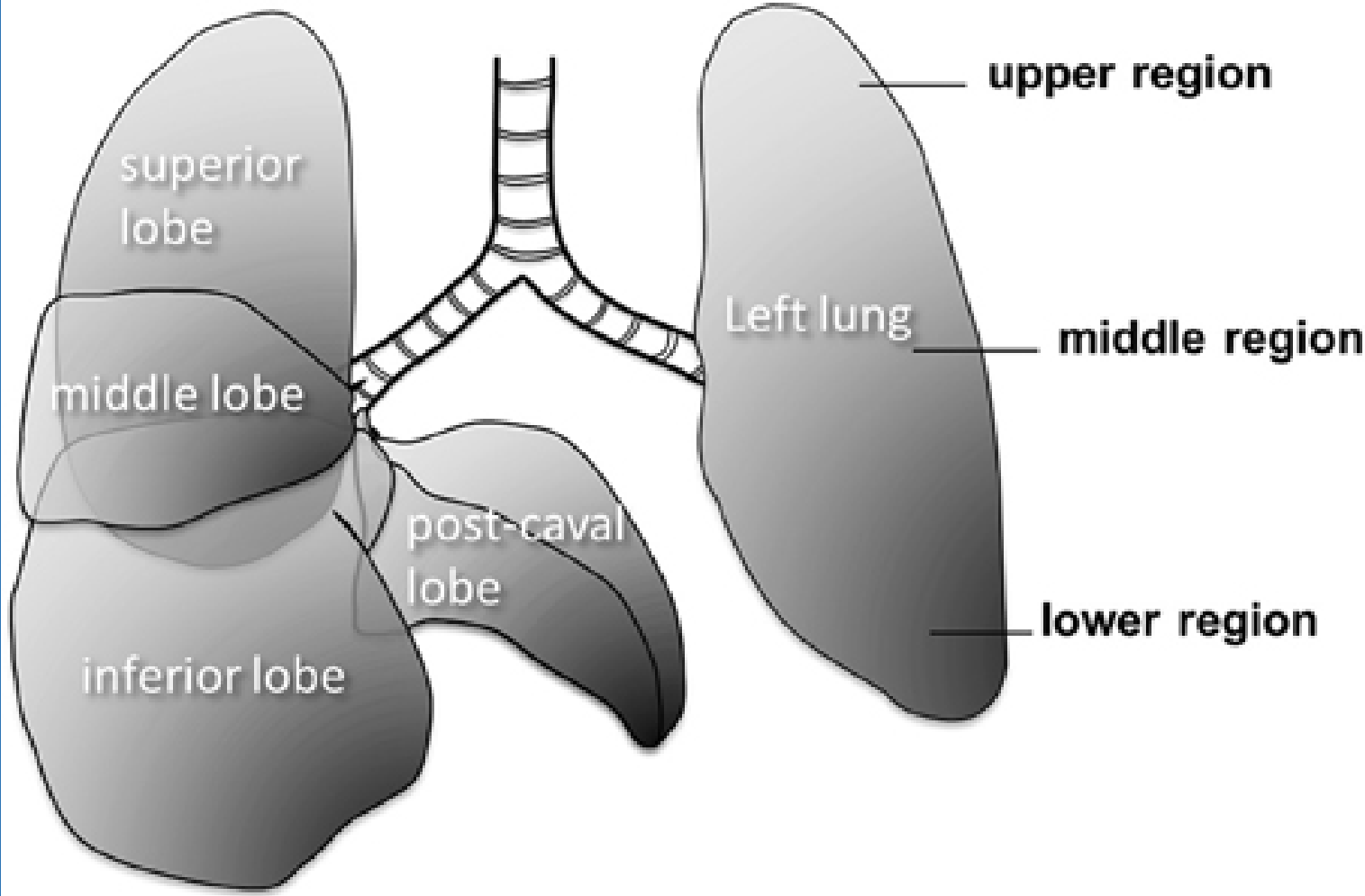


Squamous Cell Lung Cancer



Small Cell Carcinoma





Lung Cancer Treatment Algorithm

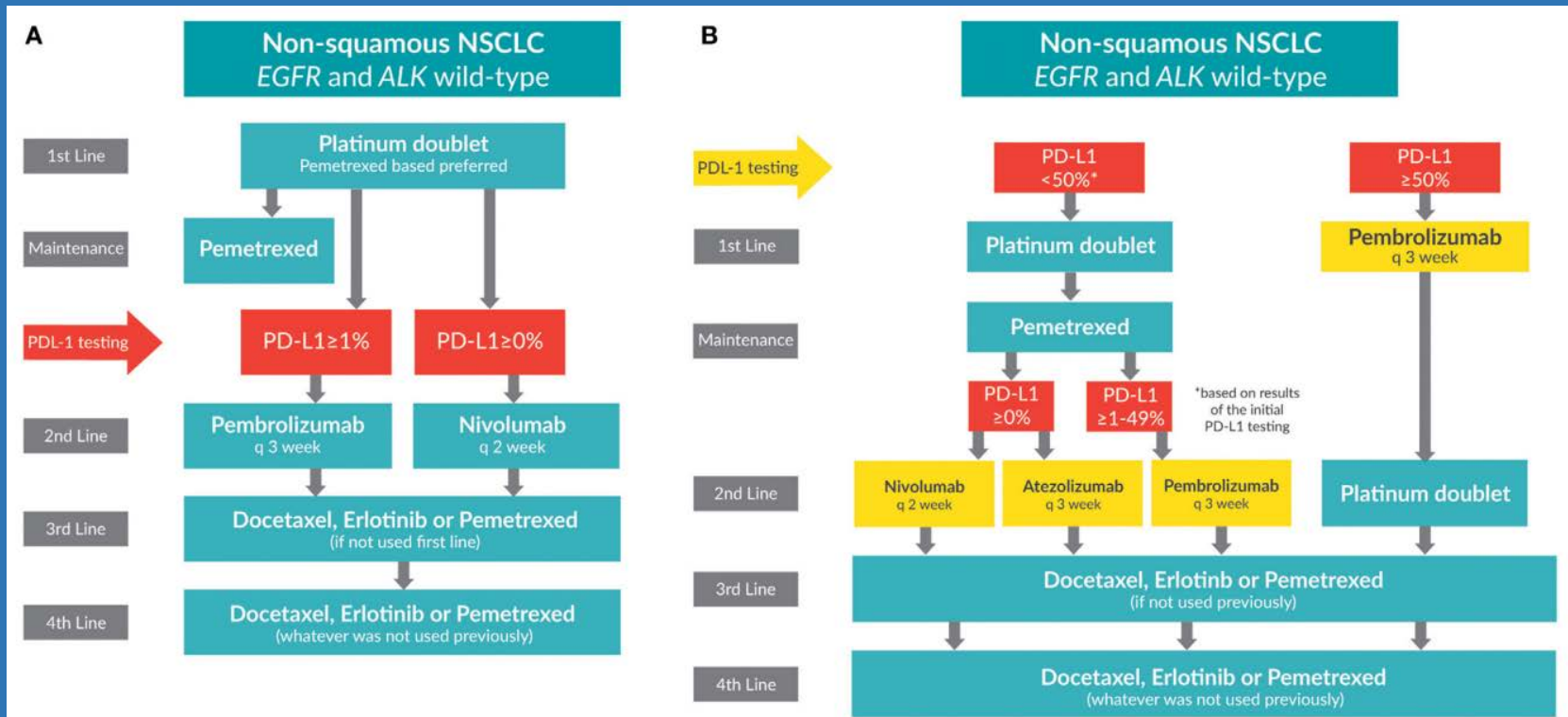
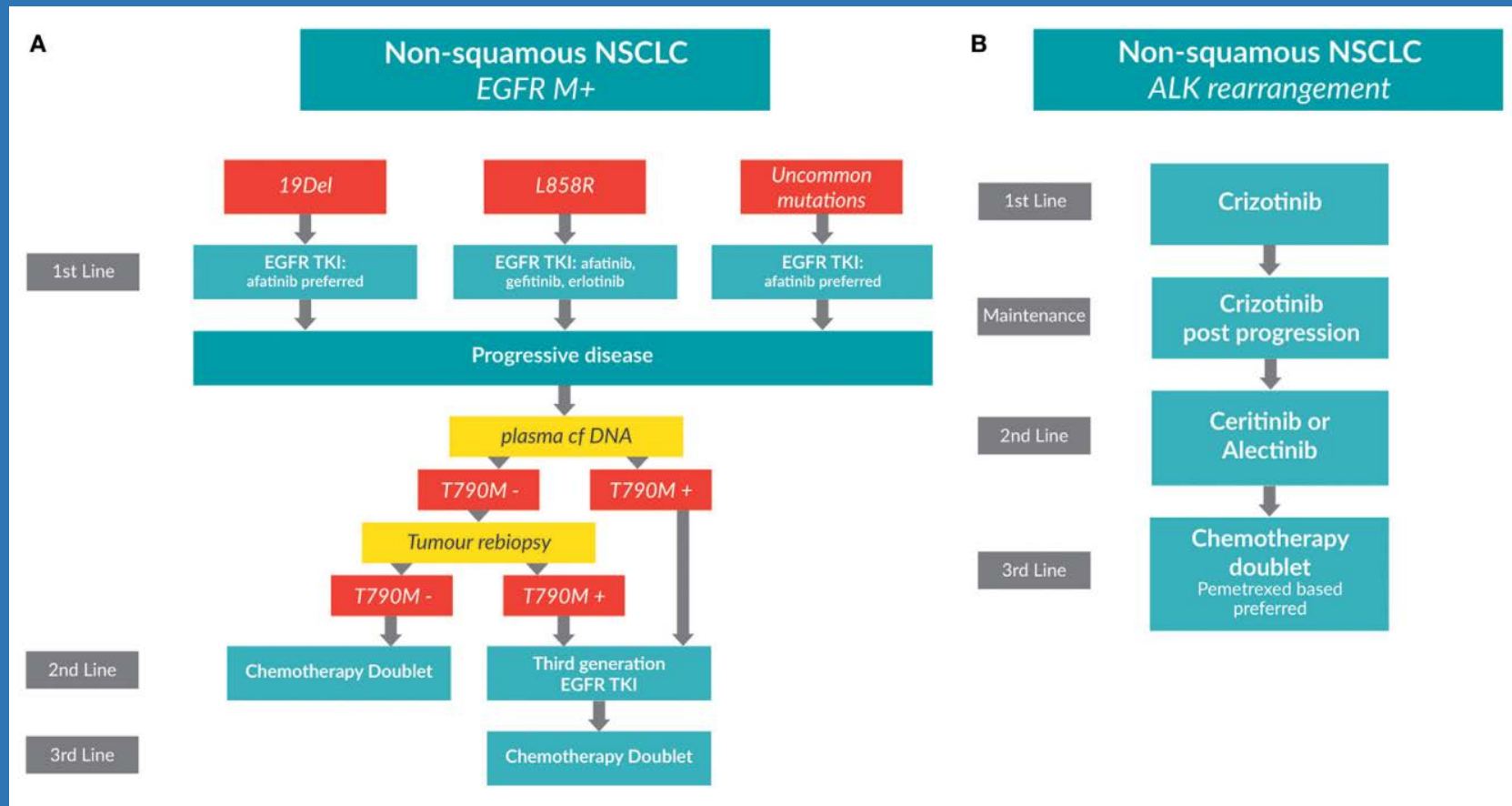


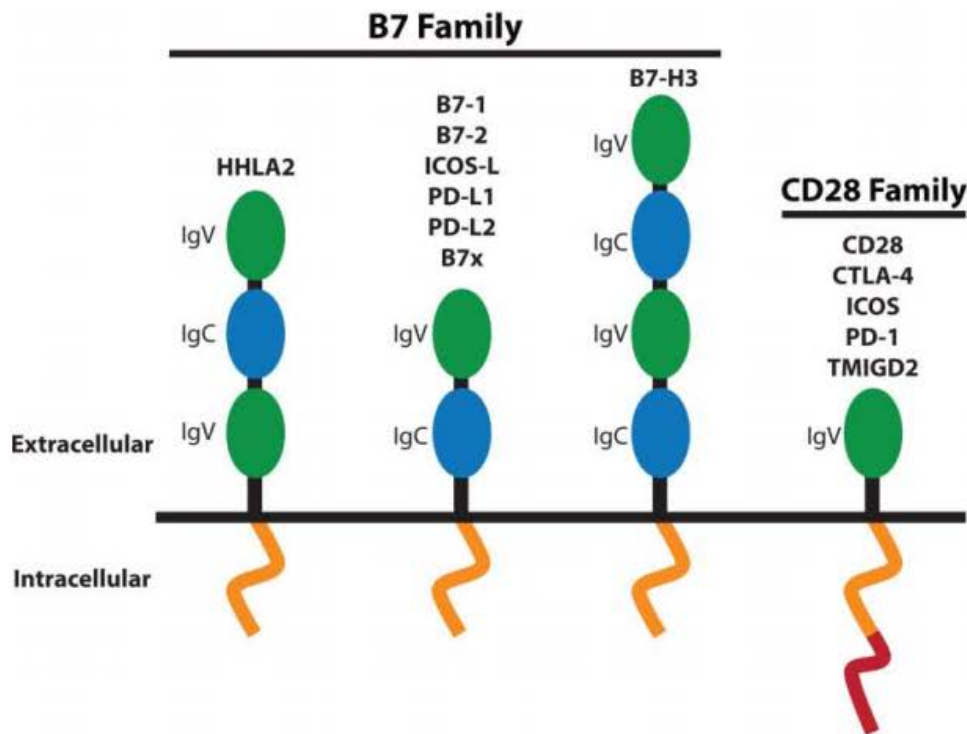
FIGURE 2 | Treatment algorithms for non-small cell lung cancer patients whose tumors do not have EGFR or ALK mutations (wild-type). (A) Current treatment algorithm. (B) Future treatment algorithm.

Lung Cancer Treatment Algorithm

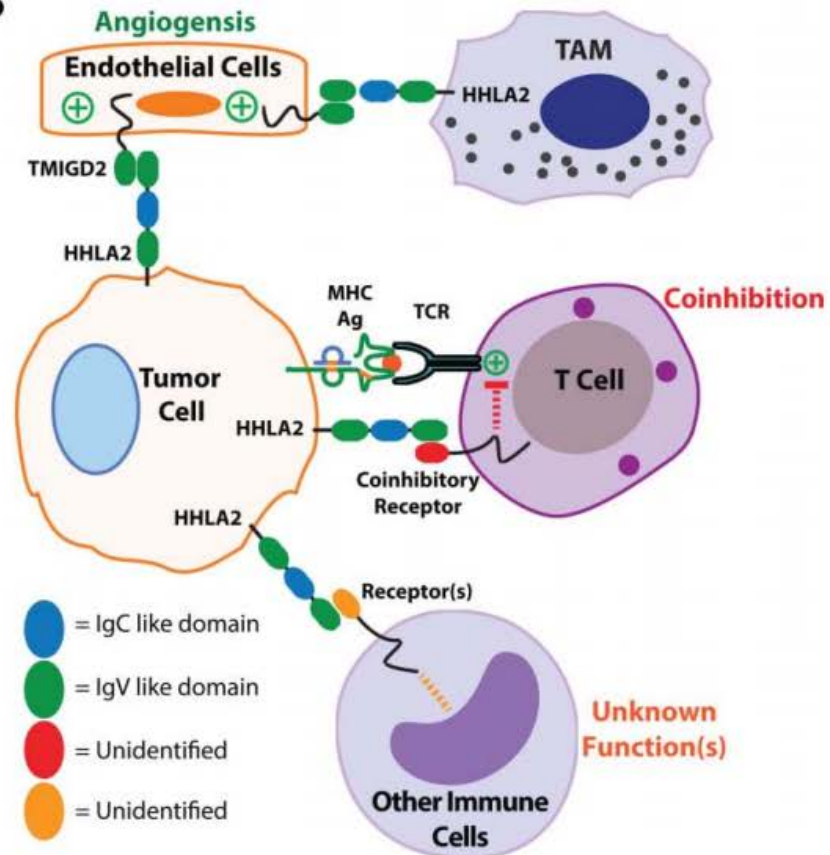


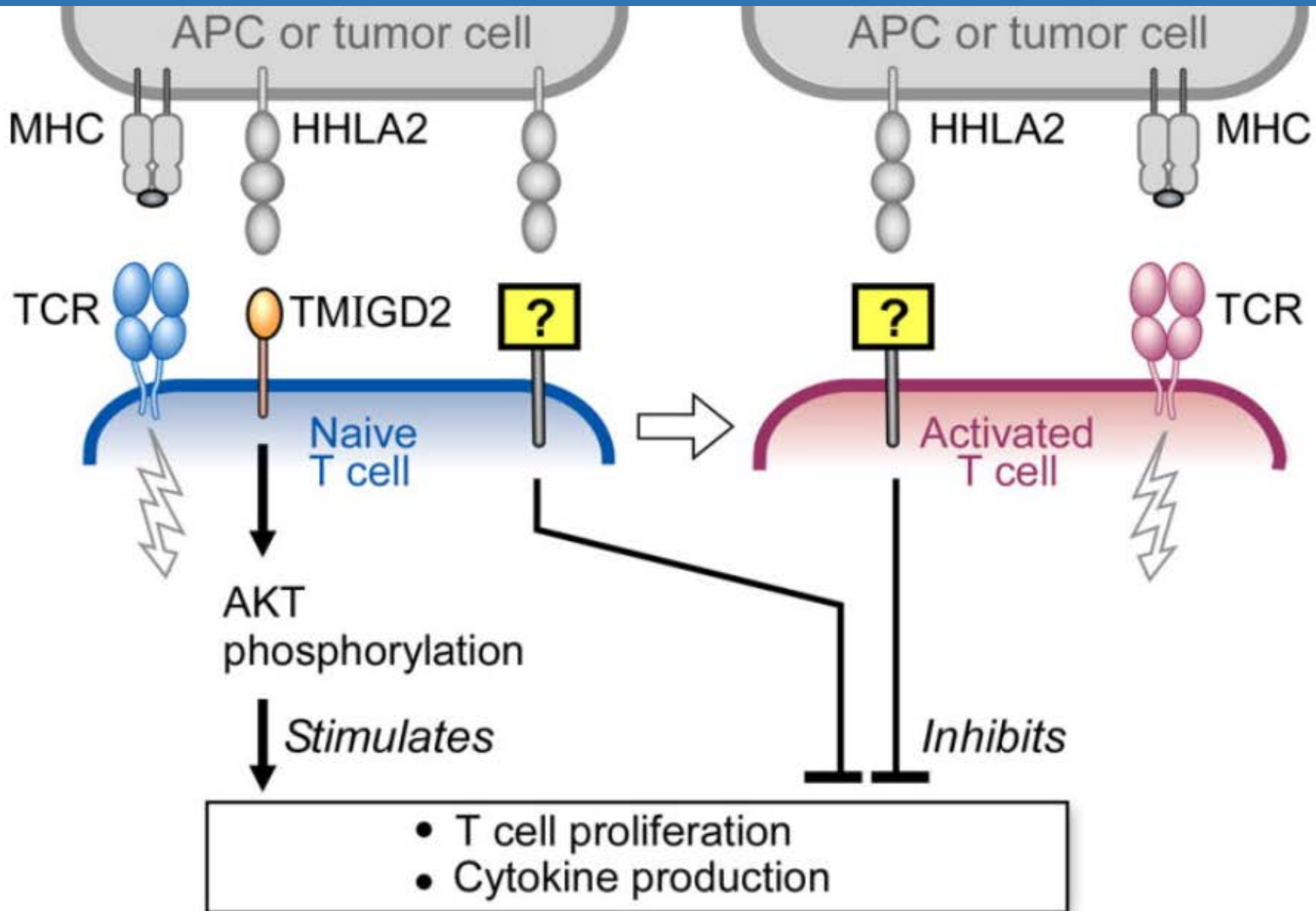
HHLA2 Signaling

A

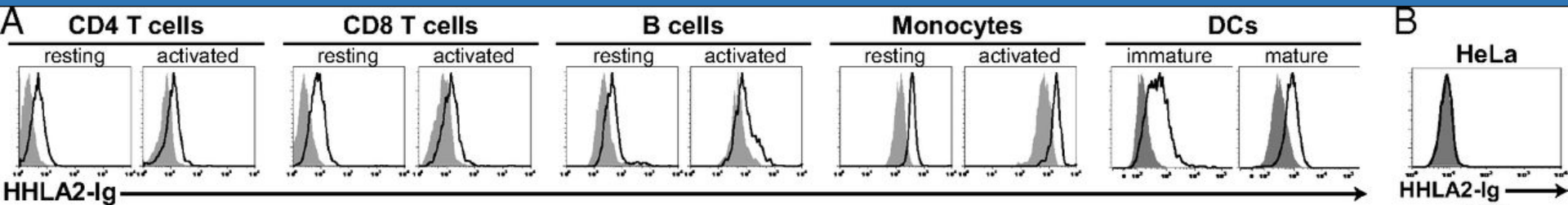


B



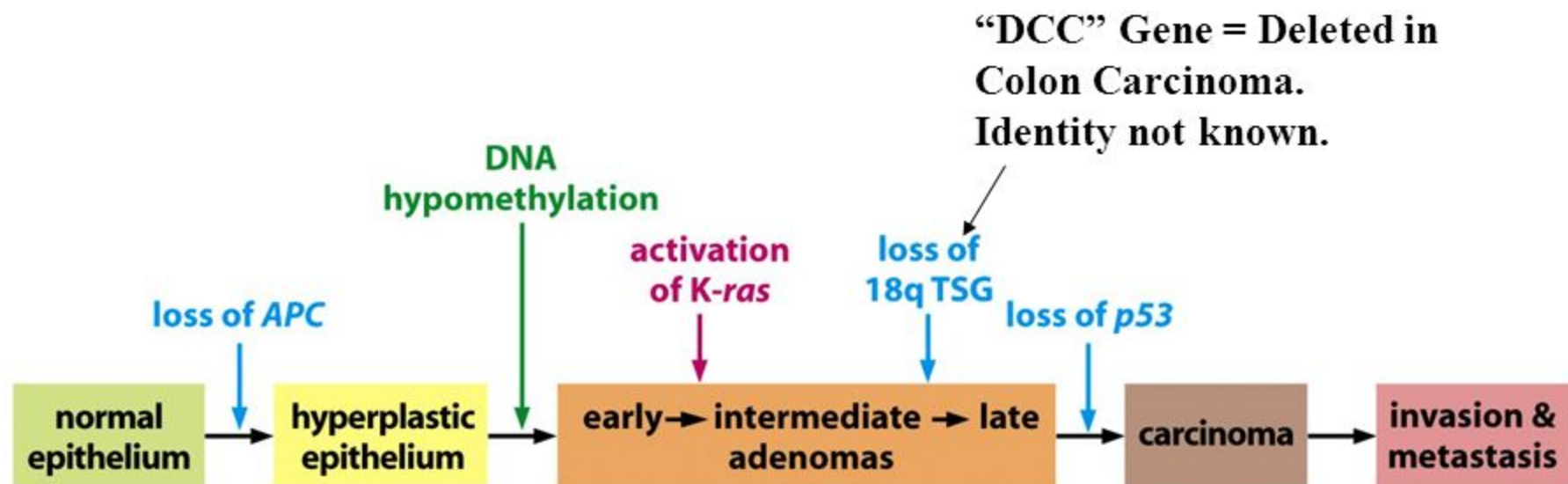


A Putative Receptor Exists for HHLA2



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

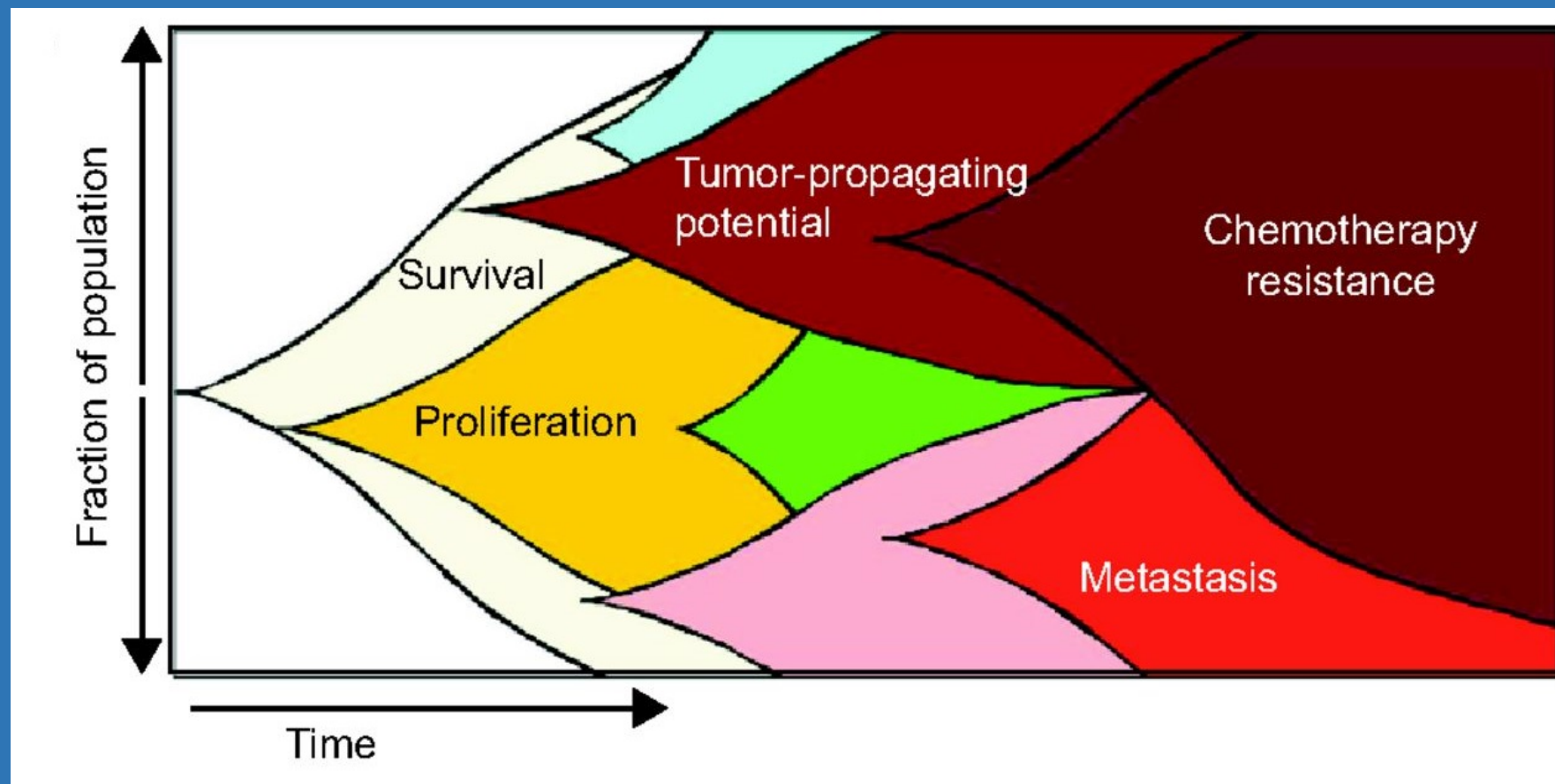
*EMBO J. 1983; 2(12): 2281–2283.
PMCID: PMC555446

Localisation of the human N-ras oncogene to chromosome 1cen - p21 by in situ hybridisation.

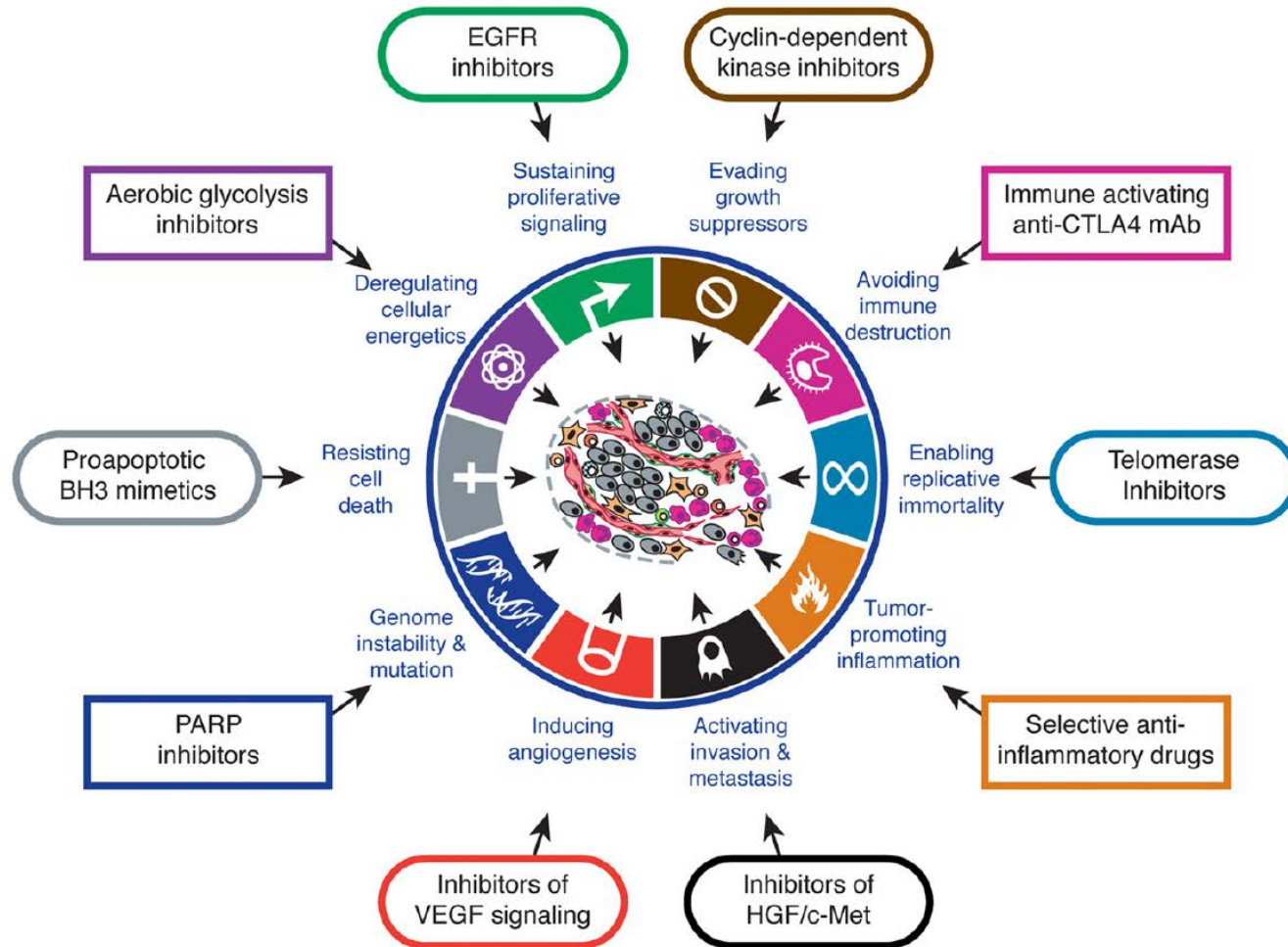
[M Davis](#), [S Malcolm](#), [A Hall](#), and [C J Marshall](#)

p. 409

Clonal Expansion of Cancer

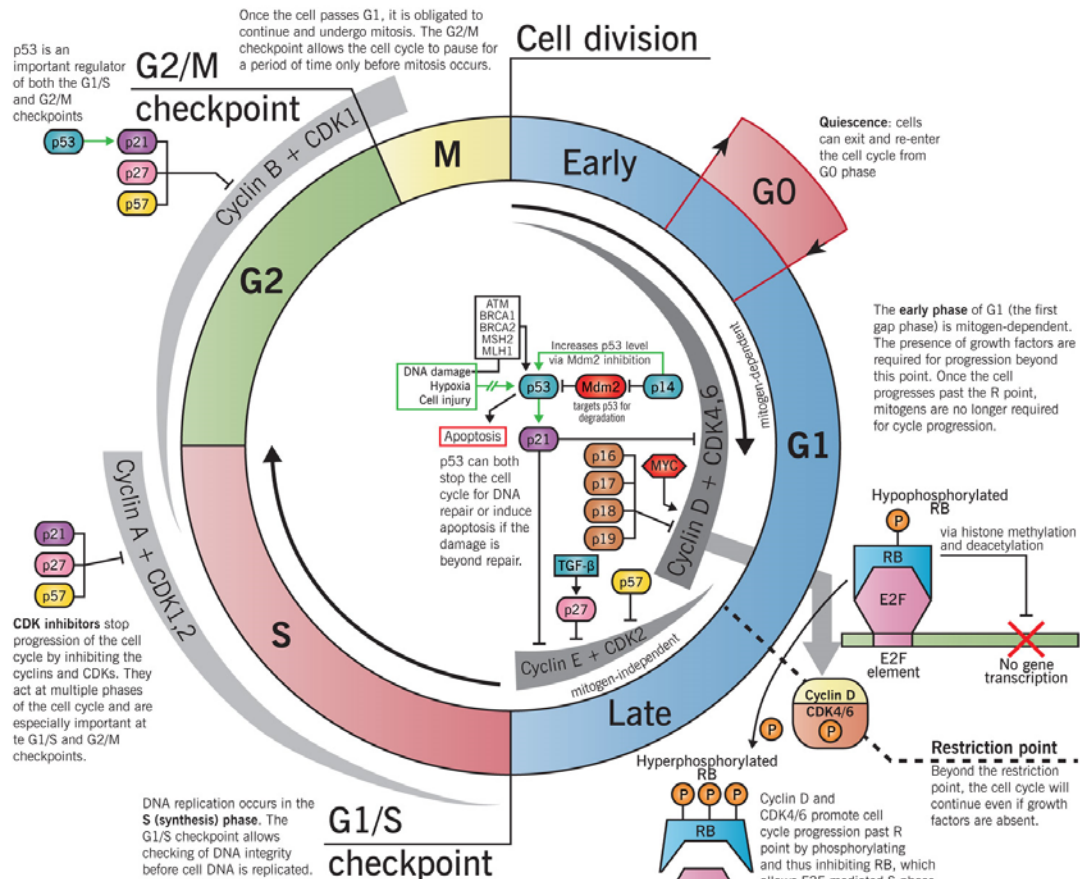


Hallmarks of Cancer



The cell cycle and implications for cancer genetics

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

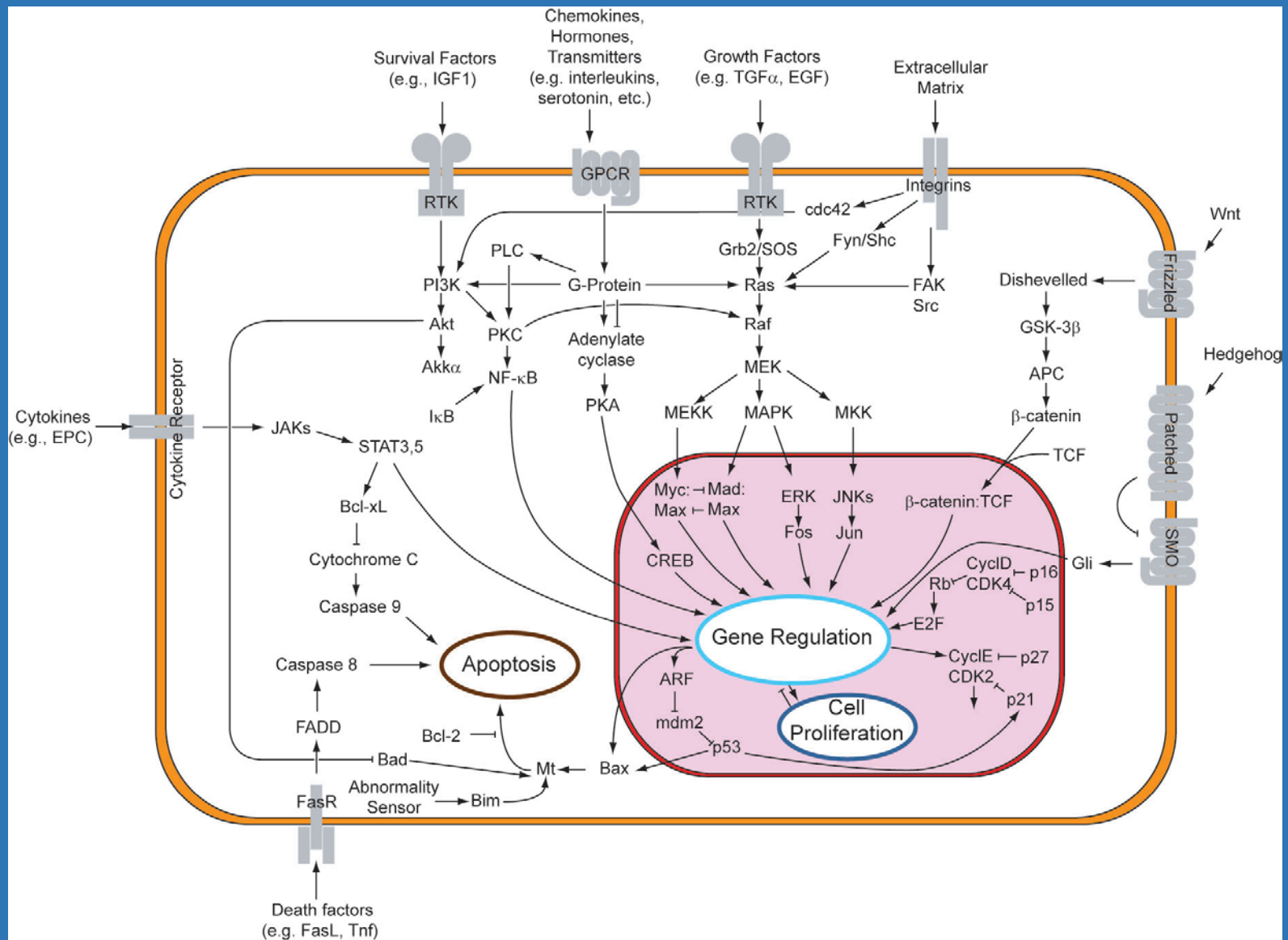
Germline mutations of *p53* gene causes **Li-Fraumeni syndrome**, in which multiple cancers develop at a relatively young age (<45). Breast cancer, osteosarcoma, leukemia, lymphoma, and adrenocortical carcinomas are the most common cancers found in Li-Fraumeni syndrome.

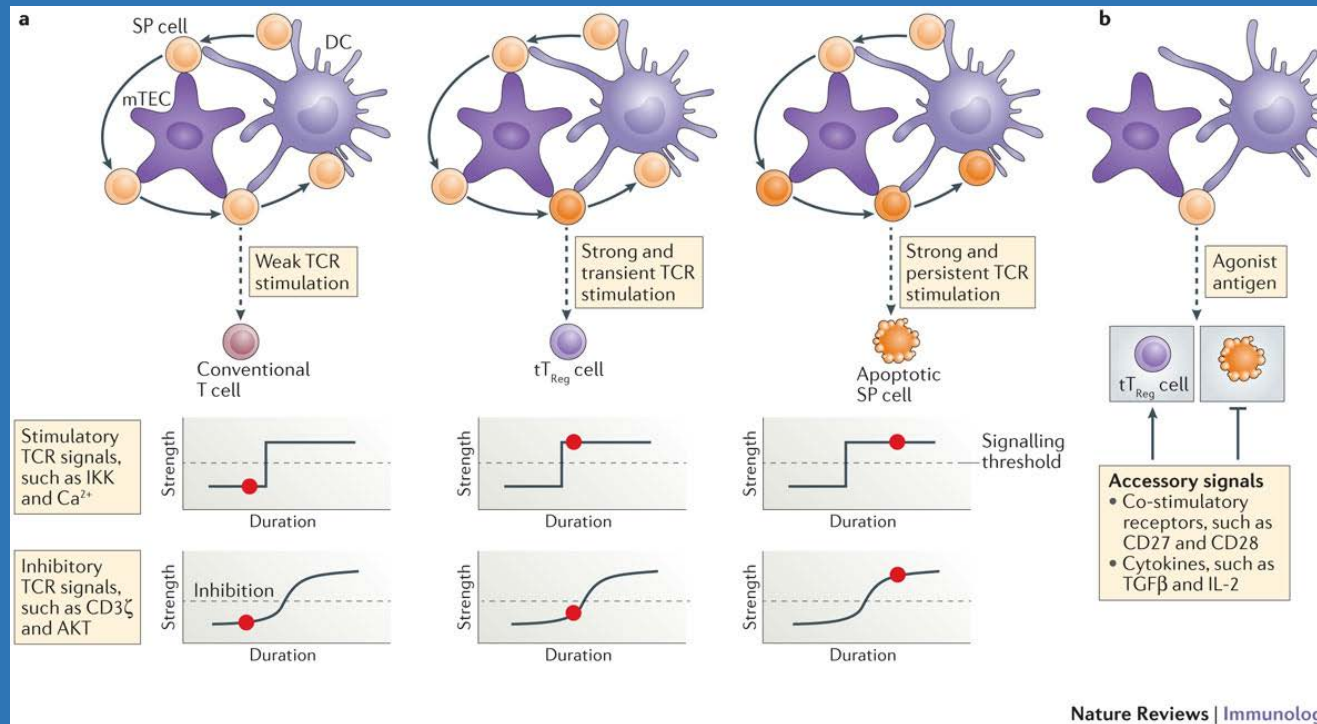
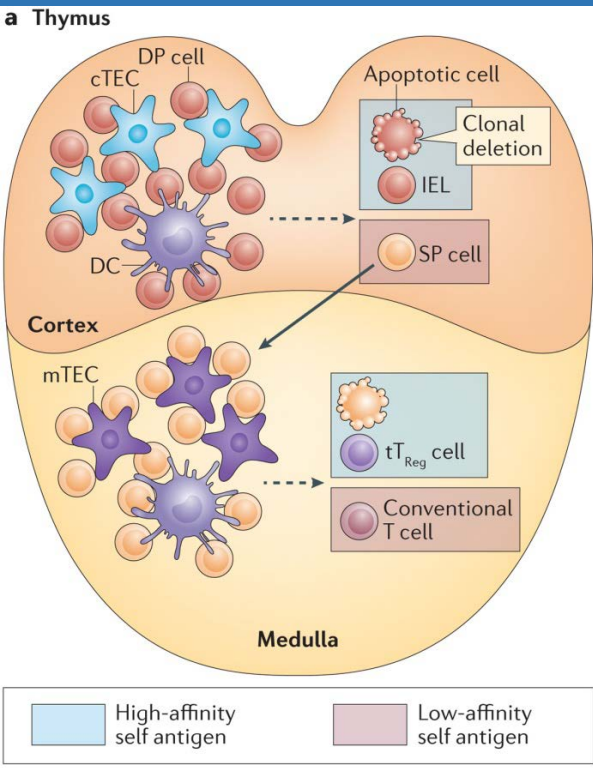
Similar to retinoblastoma, the **two-hit hypothesis** applies to Li-Fraumeni syndrome as well. One allele of *p53* is lost in the germline and the second allele is lost somatically. The risk of cancer is dominantly inherited.

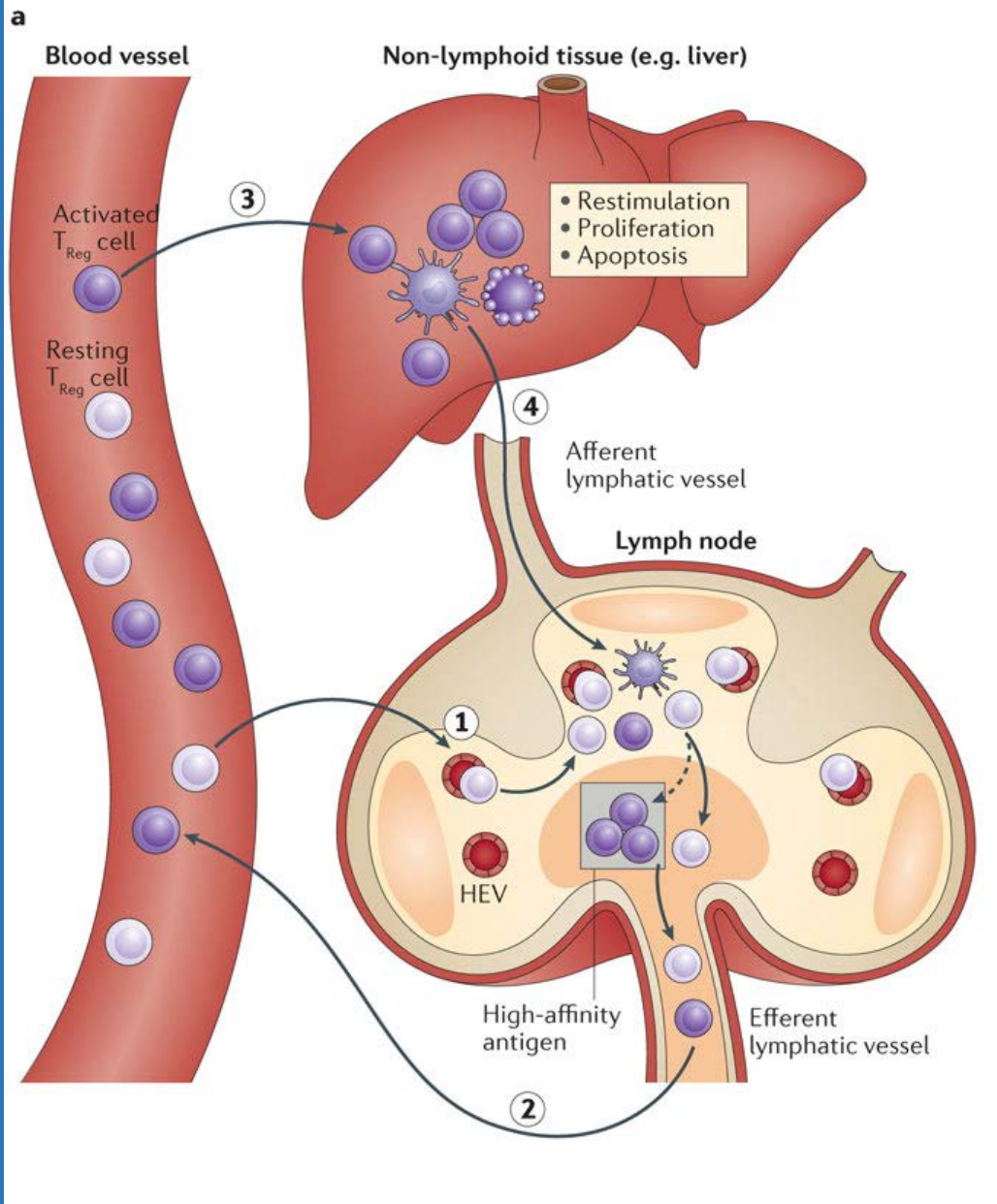
p53 is called the "guardian of the genome." It is lost in almost every type of cancer because its loss confers a significant survival advantage, leading to rapid, unchecked proliferation and evasion of apoptosis.

Oncogenes	Tumour suppressors
The lack of inhibition or gain of function of which will lead to cancer.	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

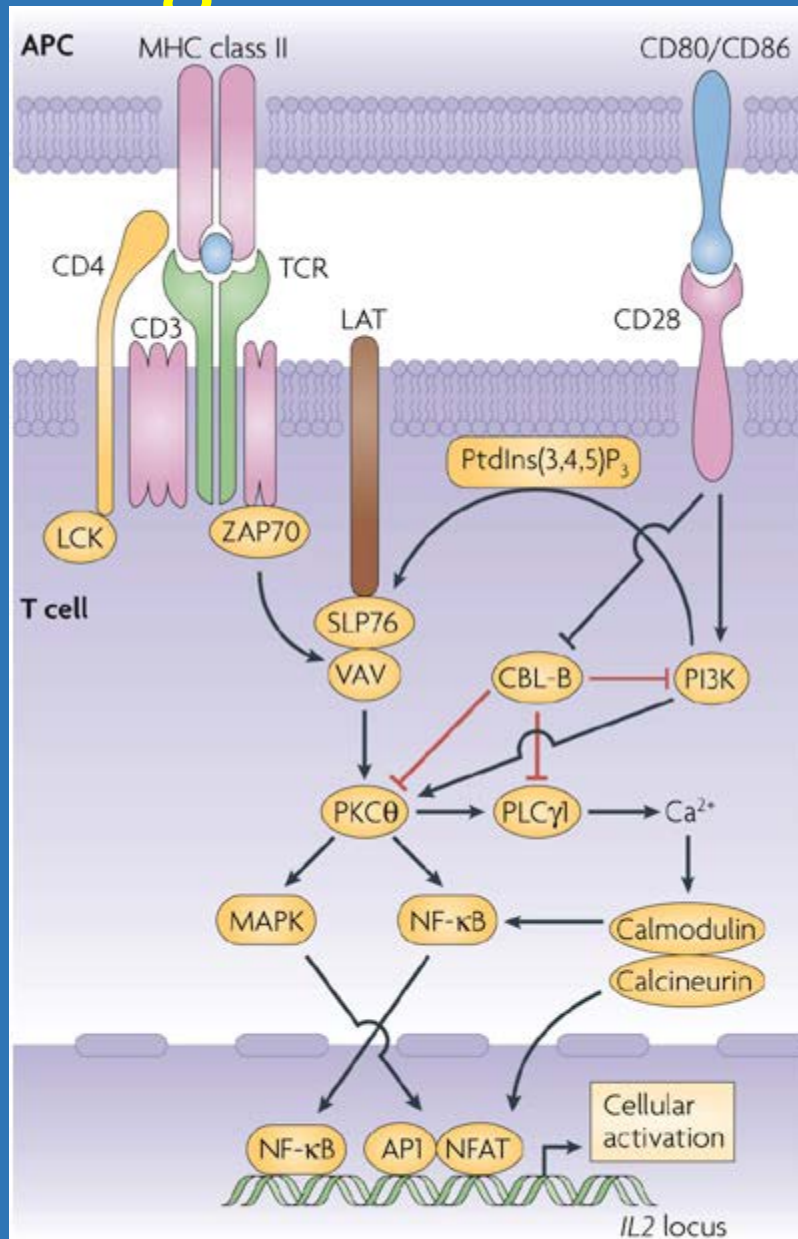
Cancer Cell Signaling





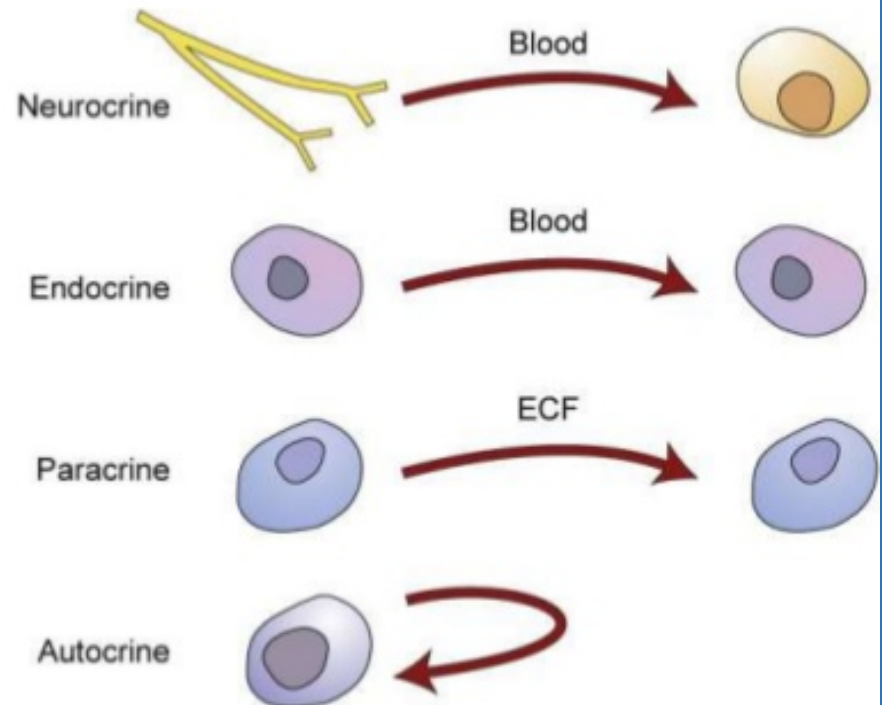


T-Cell Signal Transduction

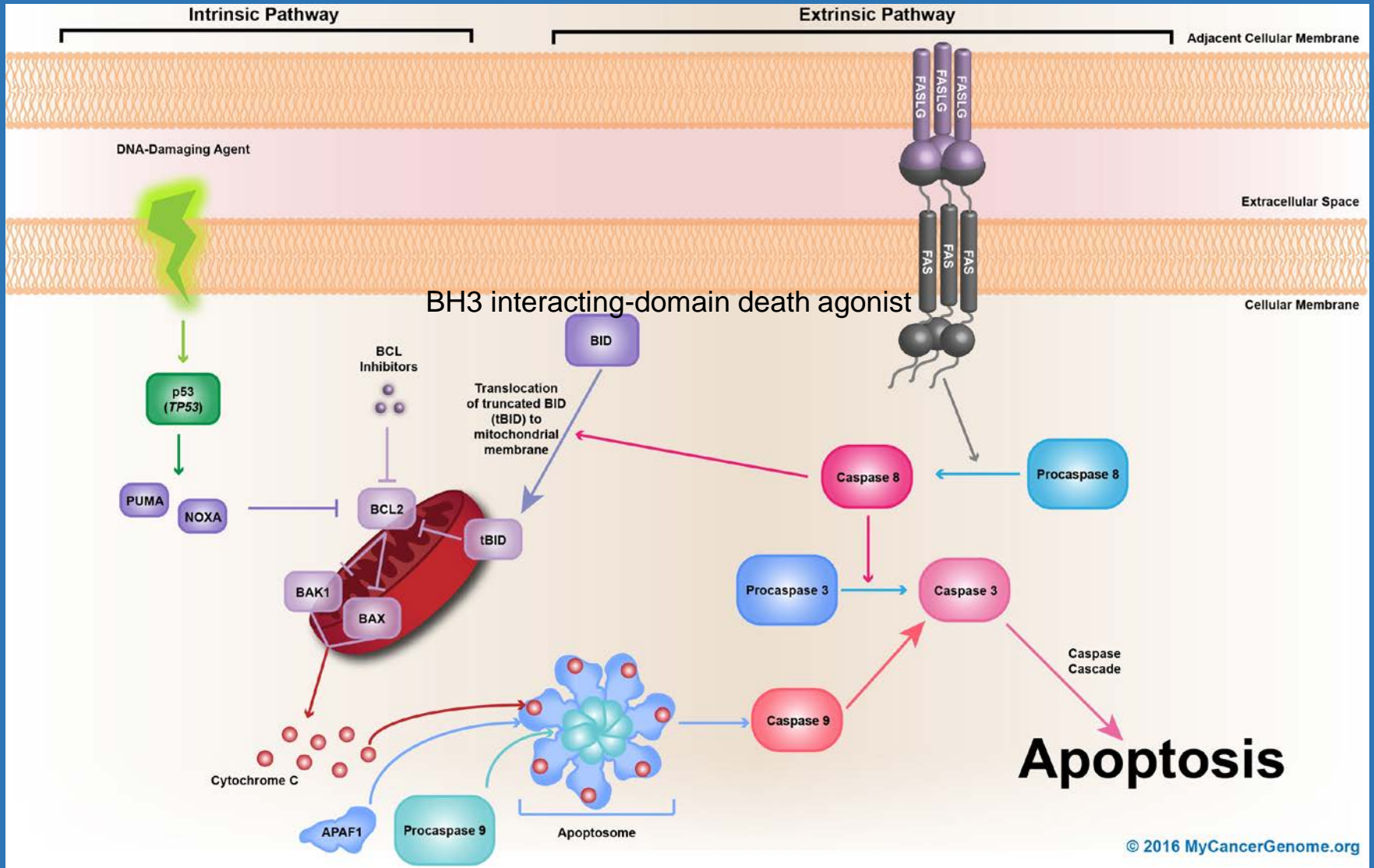


Hormones

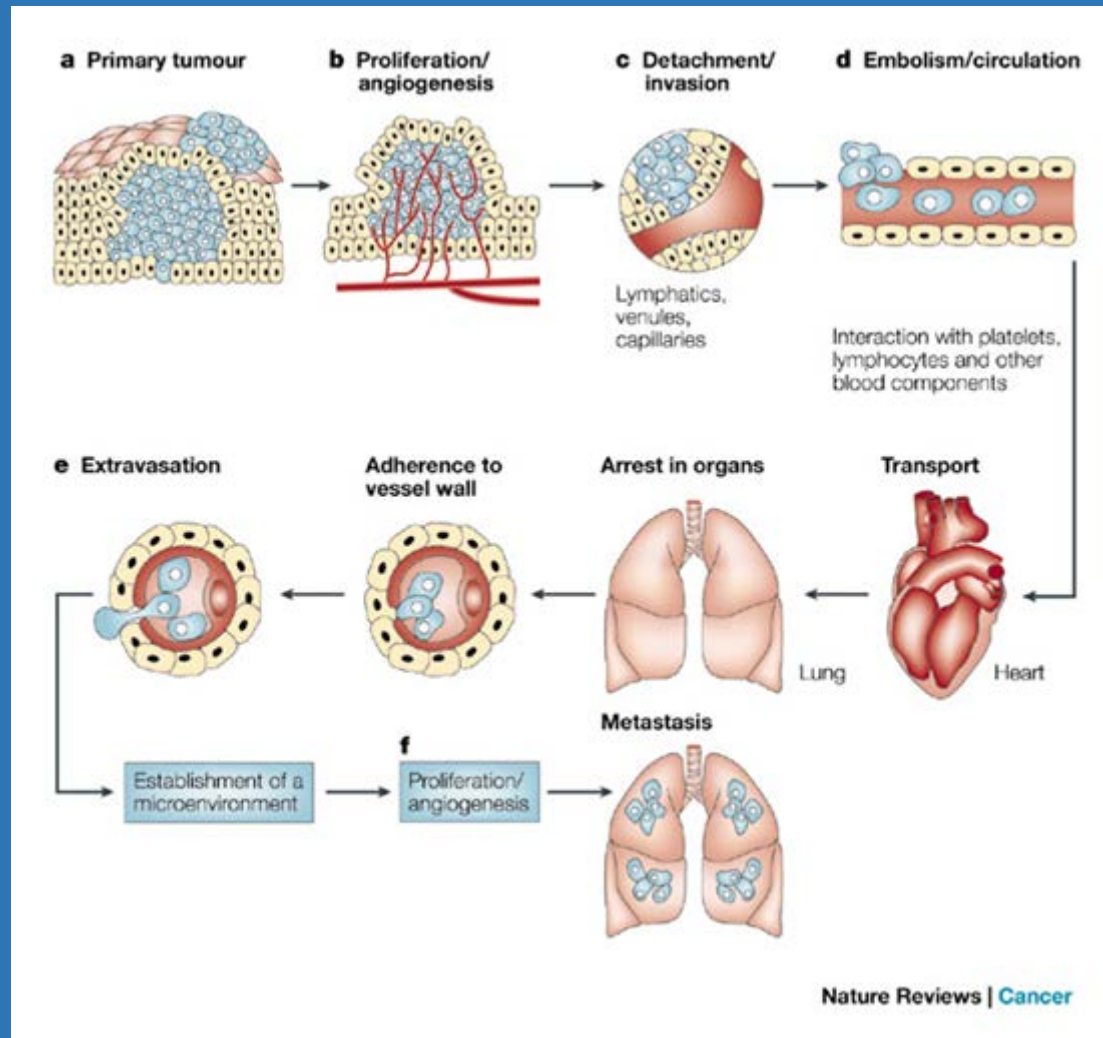
- **Neurocrine**—secretion of hormones into the bloodstream by neurons
- **Endocrine**—secretion of hormones into the bloodstream by endocrine glands
- **Paracrine**—hormone molecule secreted by one cell affects adjacent cells
- **Autocrine**—hormone molecule secreted by a cell affects the secreting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



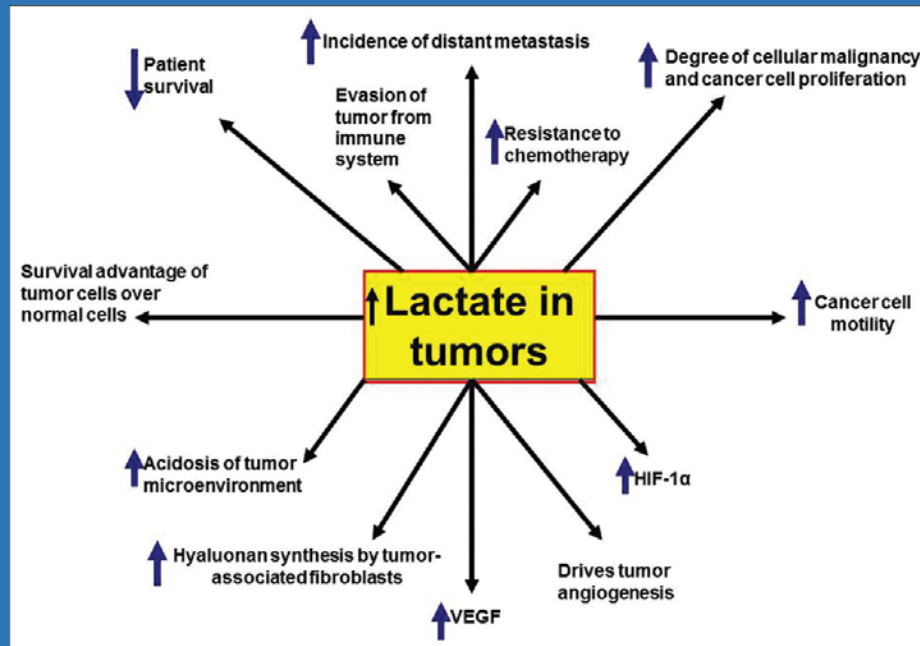
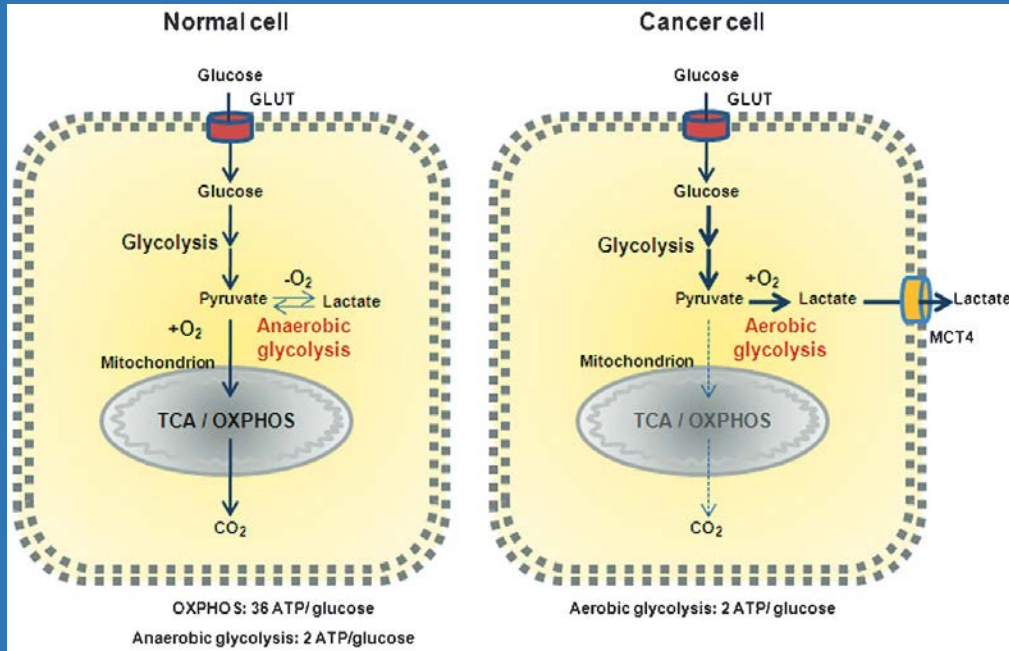
APOPTOSIS



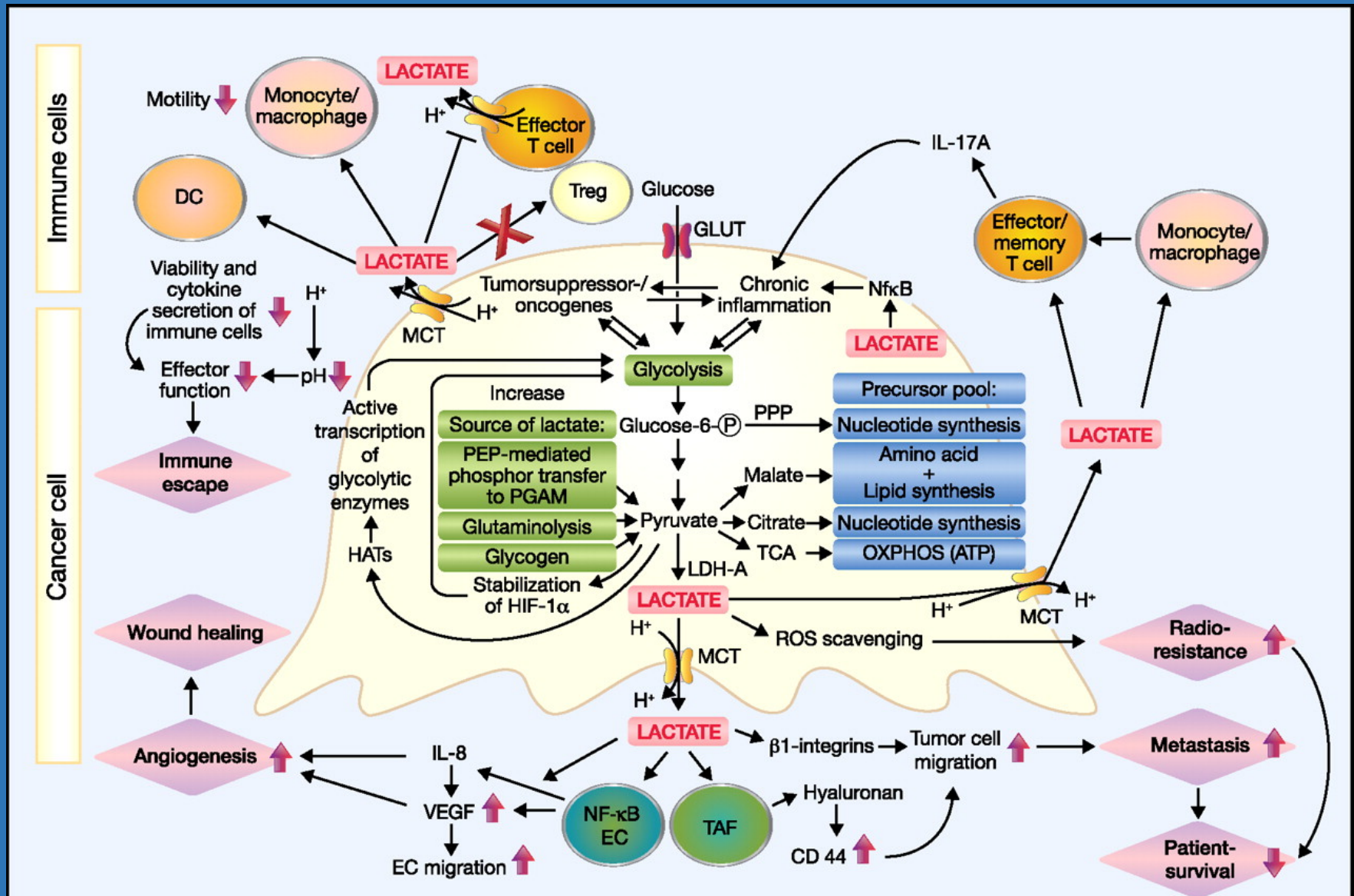
Invasion Metastasis Cascade



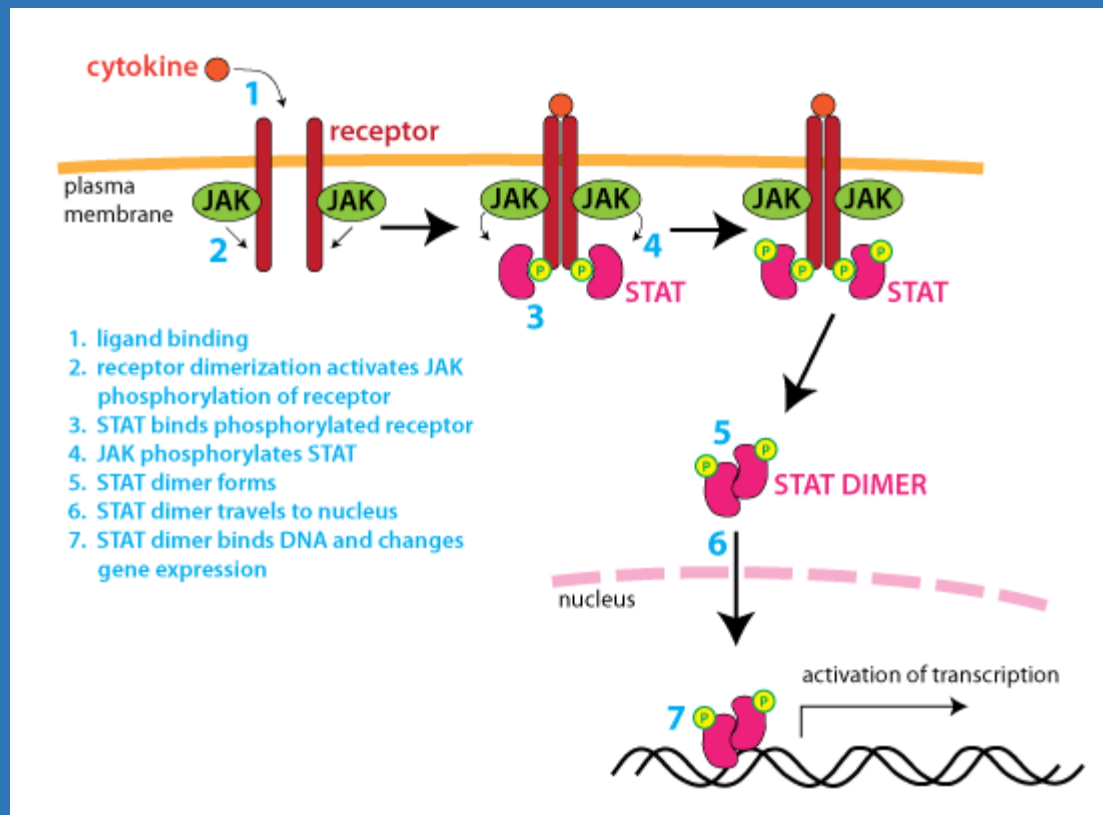
Cancer Cell Metabolism



Cancer Cell Metabolism



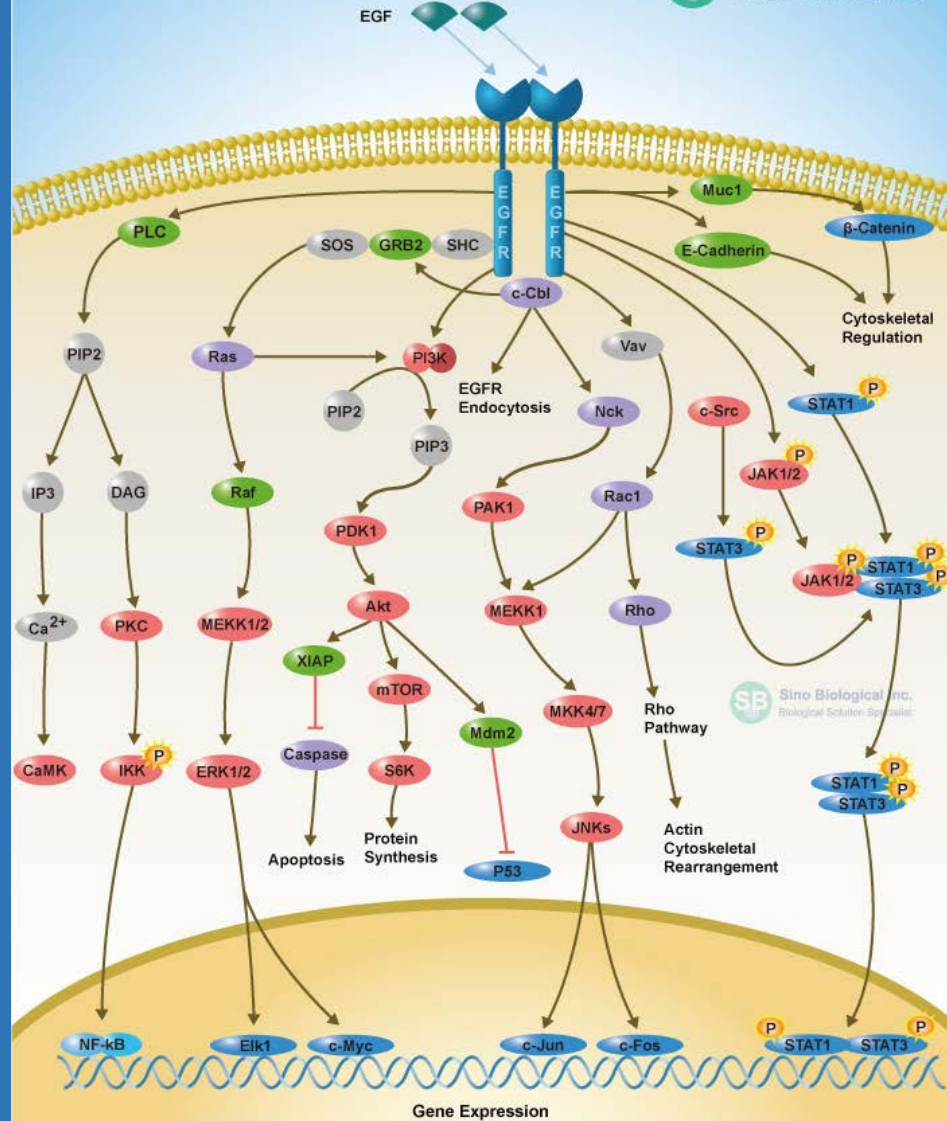
Jak/Stat Signalling



EGFR Signaling

EGFR Signaling

SB Sino Biological Inc.
Biological Solution Specialist



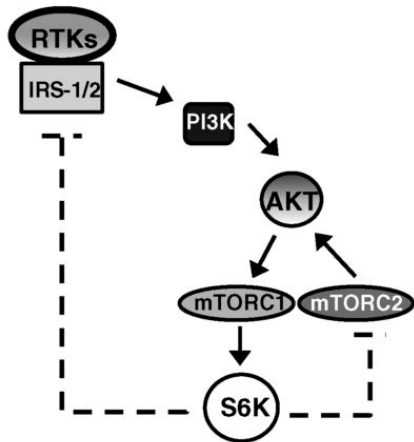
Sino Biological provides scientists around the world with quality protein, antibody, ELISA kit, cDNA

www.sinobiological.com

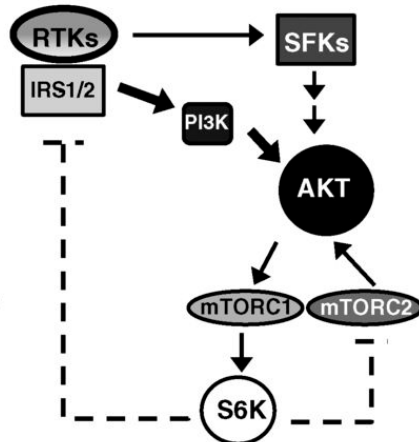
Src Signaling

Src Family kinase

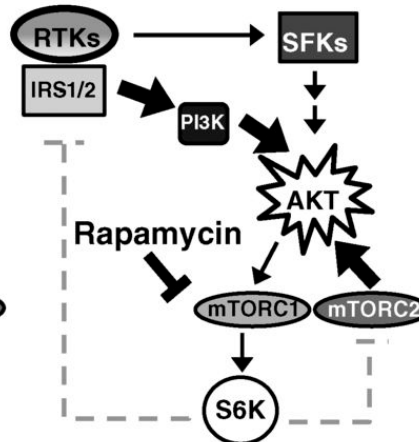
A Intact mTOR signaling
Normal cell



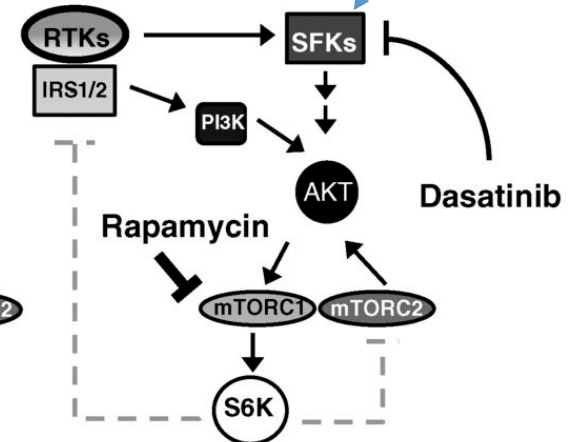
B Constitutive signaling
Tumor cell



C Rapamycin-treated
Tumor cell

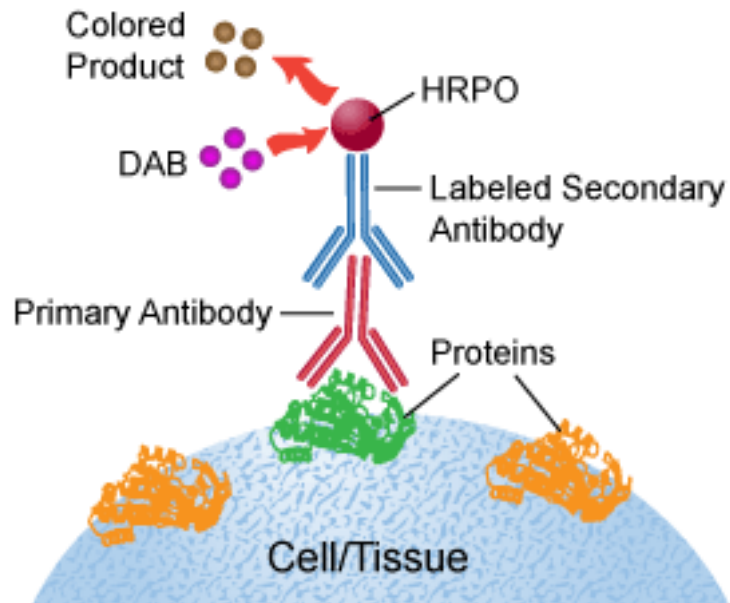


D Rapamycin + dasatinib
treated tumor cell



IHC

Indirect Immunohistochemistry



Immunofluorescence

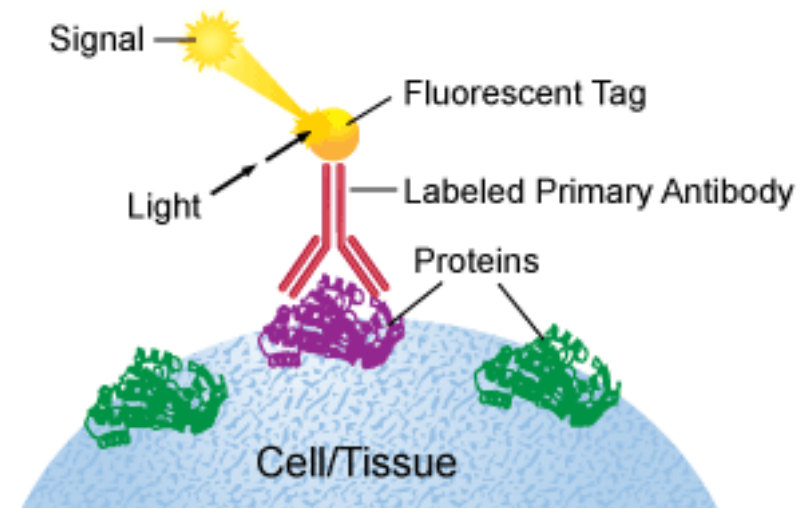
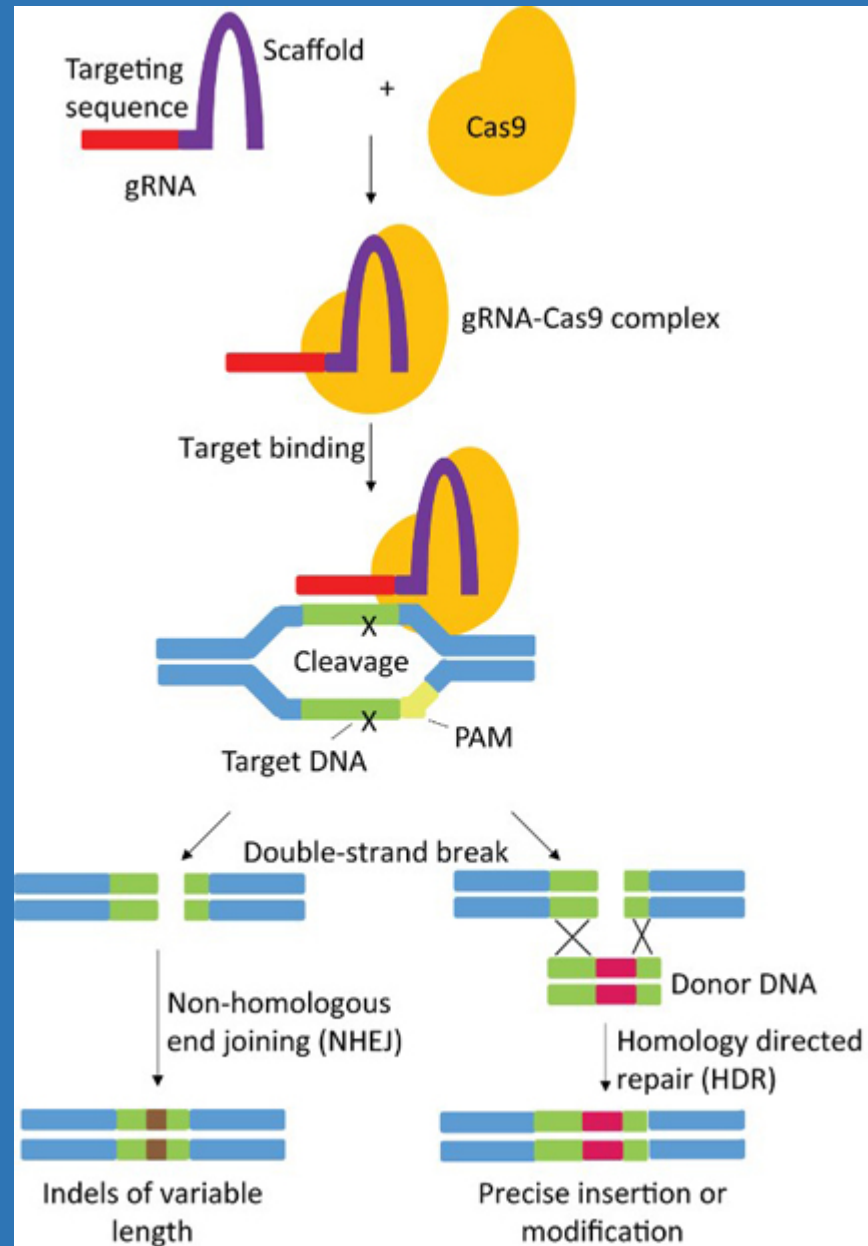


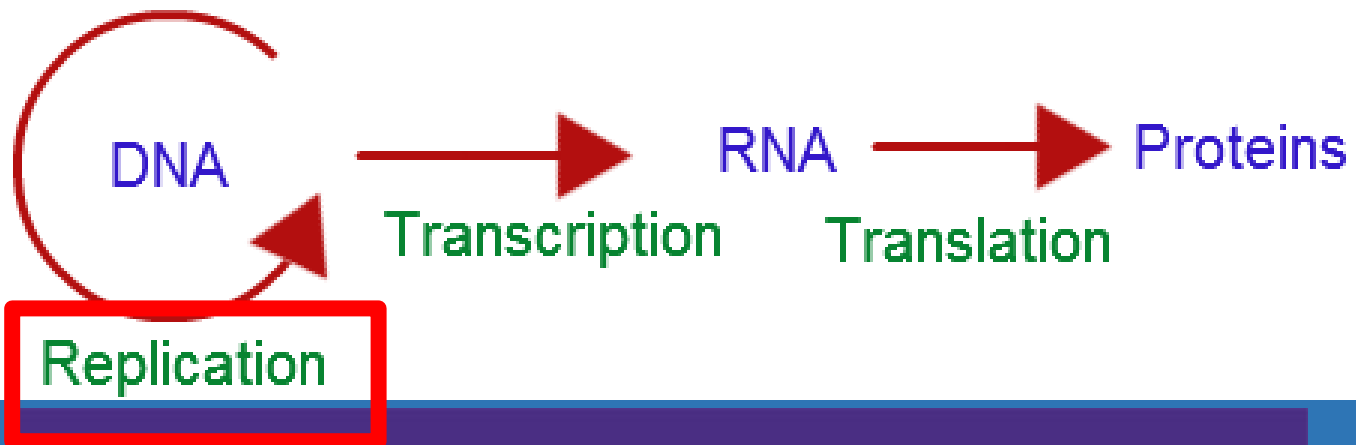
Diagram 1: Illustration of Indirect Immunohistochemistry and Immunofluorescence methods.

CRISPR/Cas9

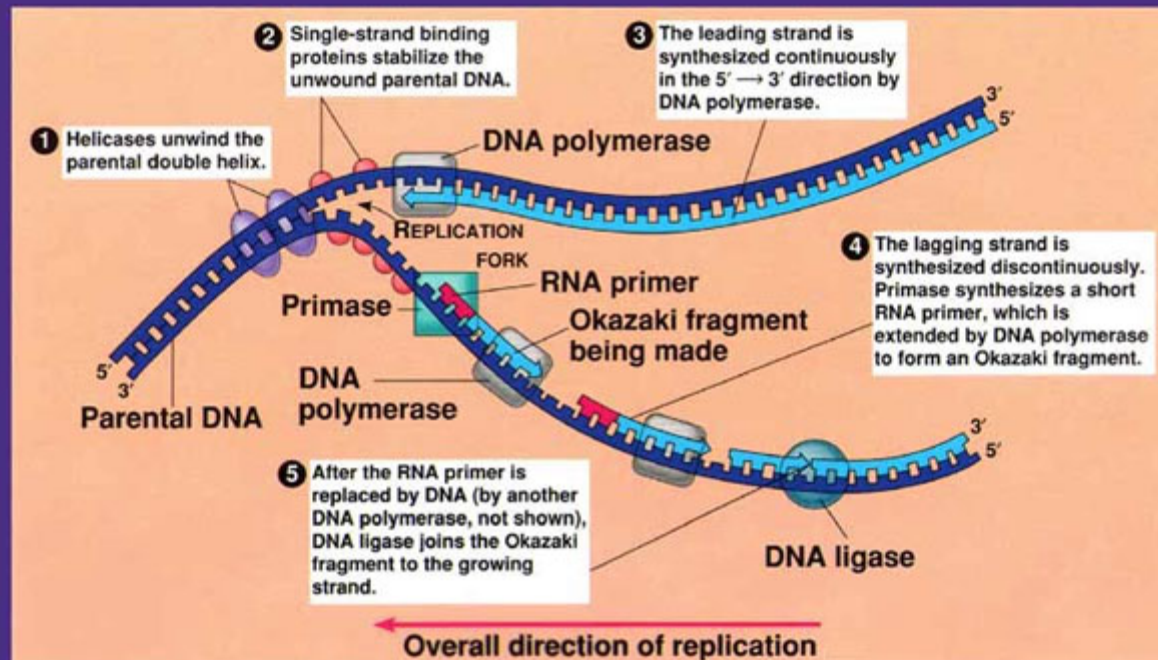


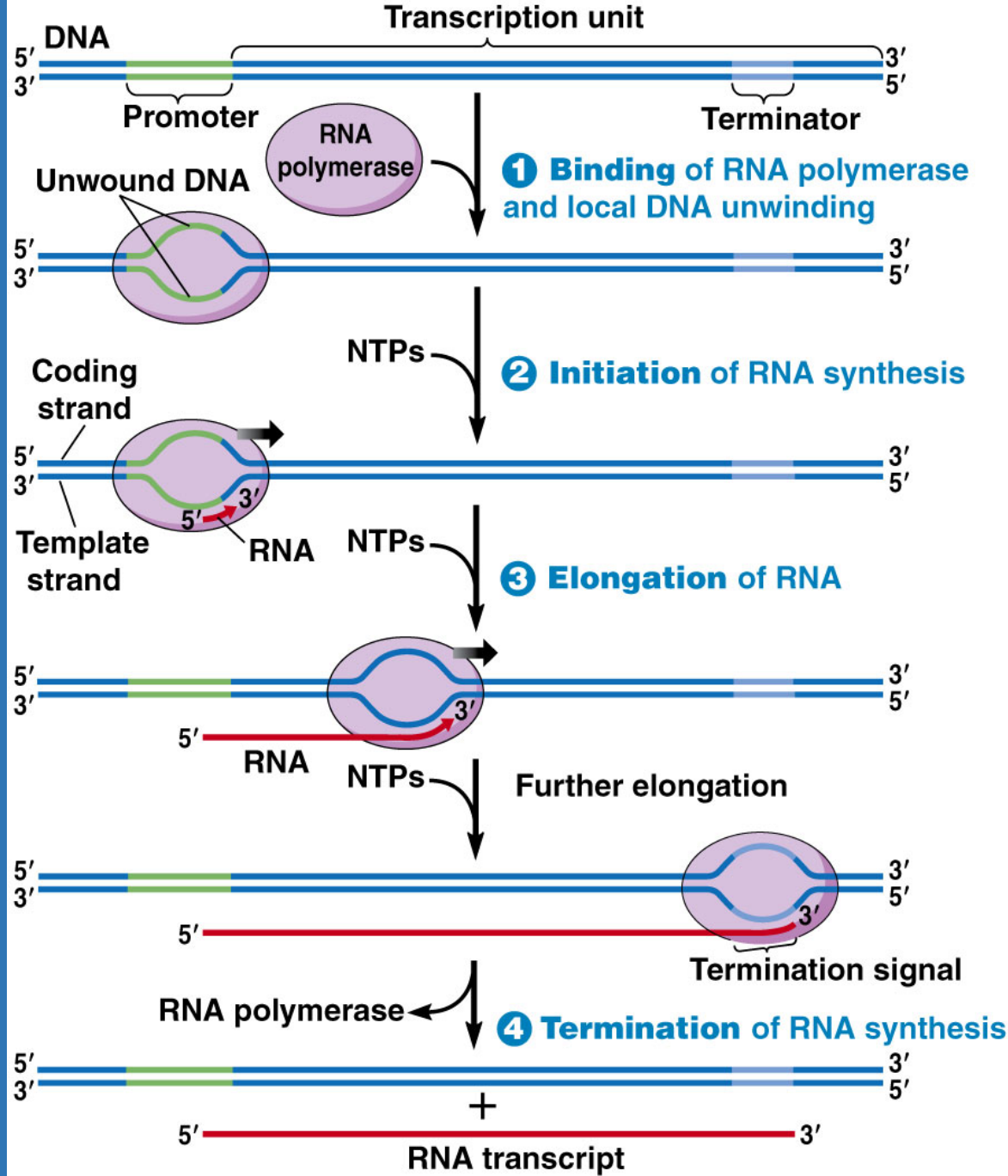
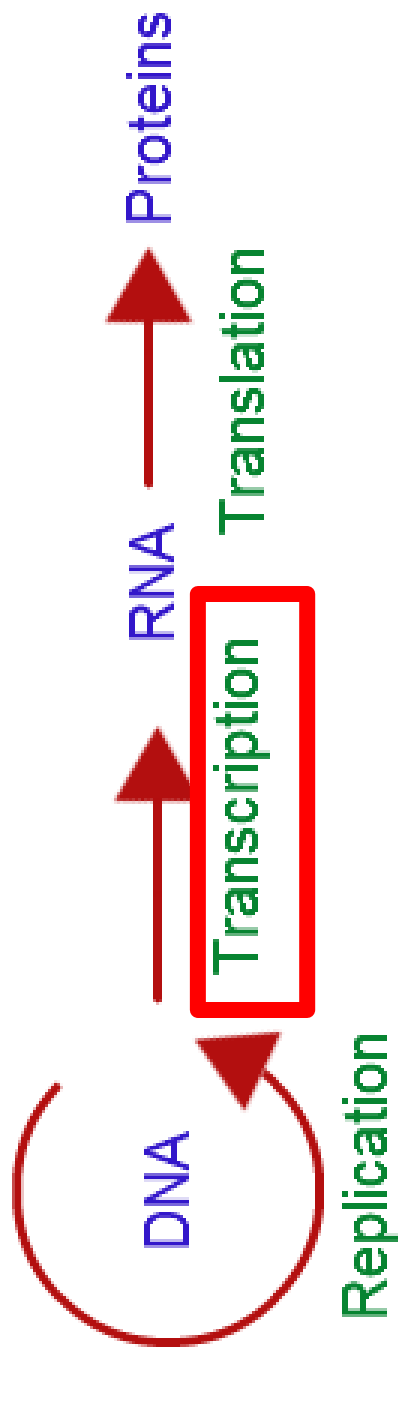
Immunotherapy for NSCLC

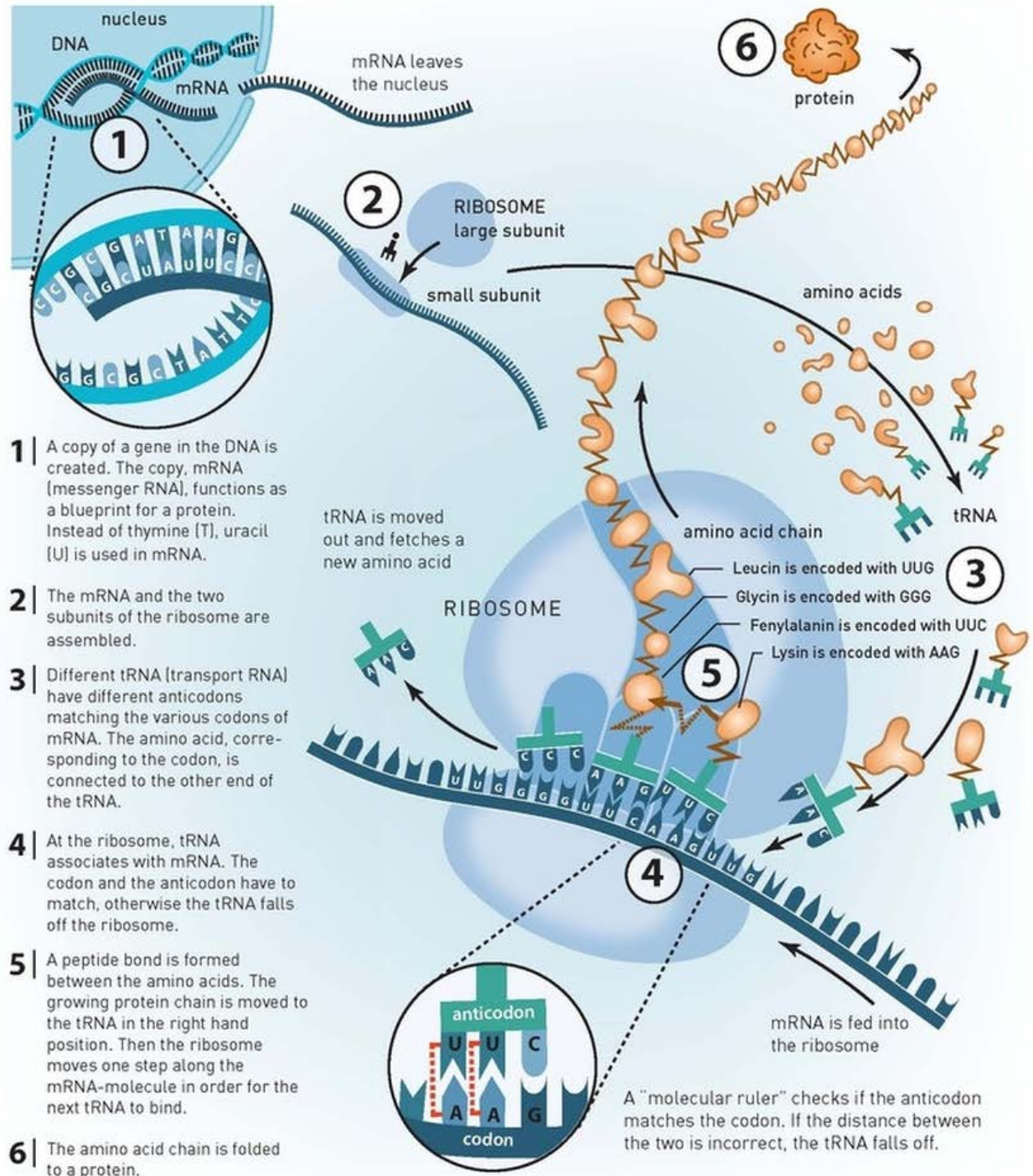
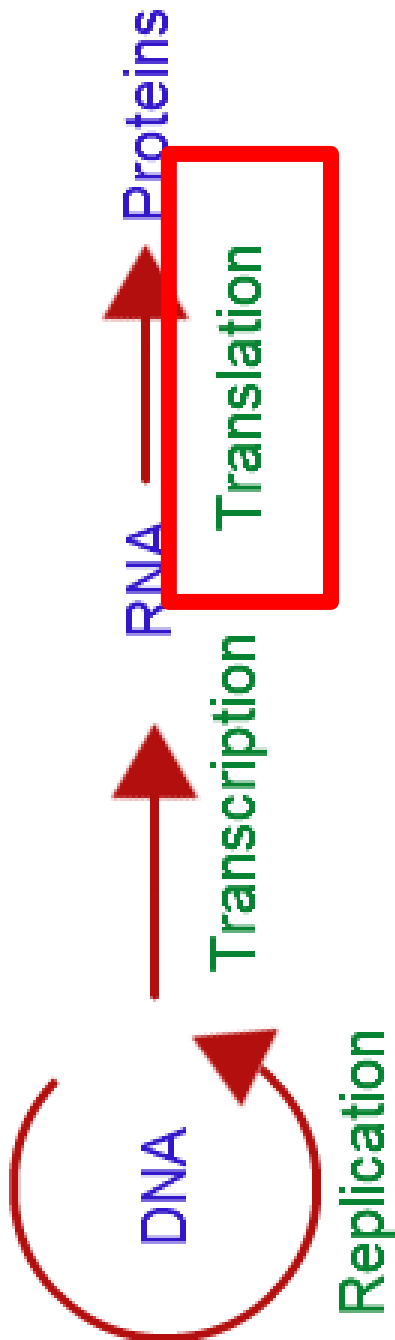
- Atezolizumab (aPD-L1), 2016 approval
 - metastatic NSCLC that has progressed during or after first-line chemotherapy with a platinum-based drug.
 - 12.6 vs. 9.7 (docetaxel)
 - Side effects were less frequent
- Pembrolizumab (aPD-L1), 2016 approval
 - the first-line treatment in PD-L1 expressing metastatic 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



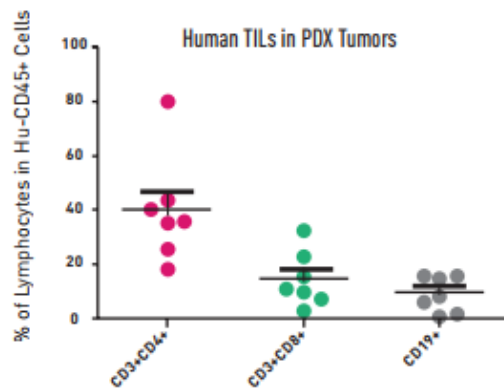
A SUMMARY OF DNA REPLICATION



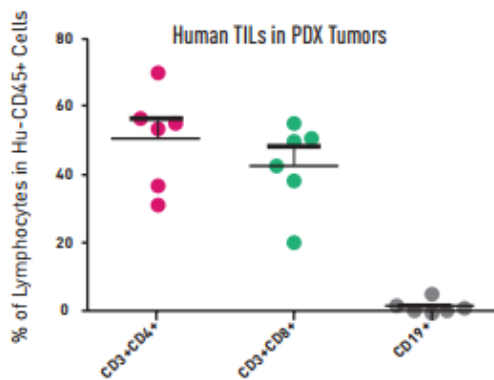




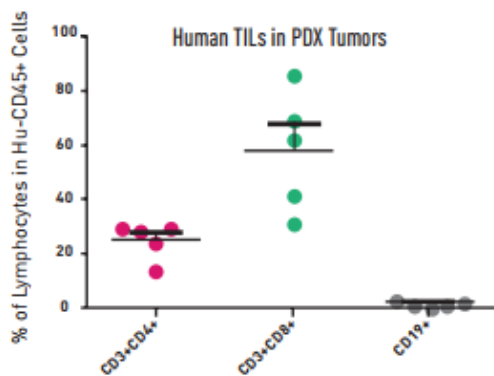
A. Breast



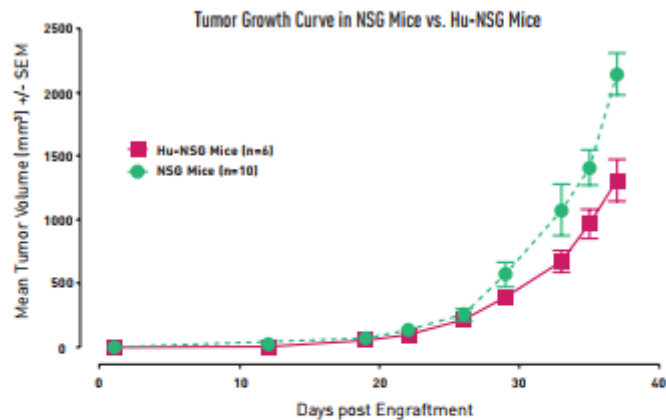
B. Lung



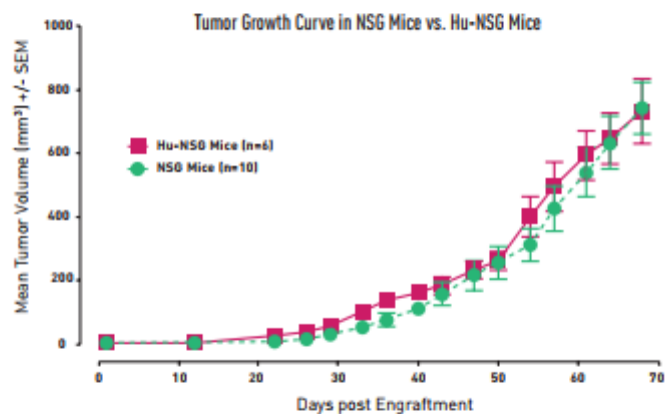
C. Sarcoma



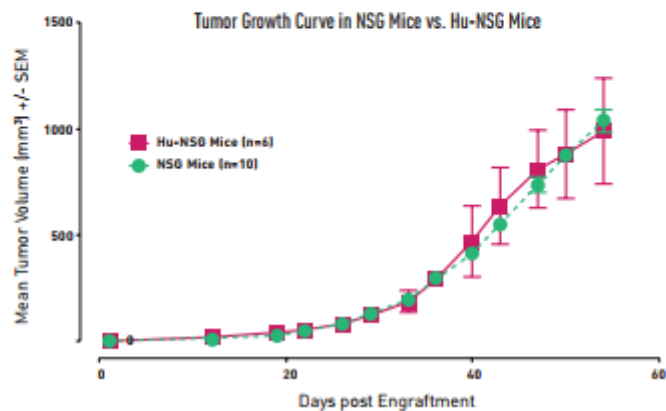
A. Breast



B. Lung

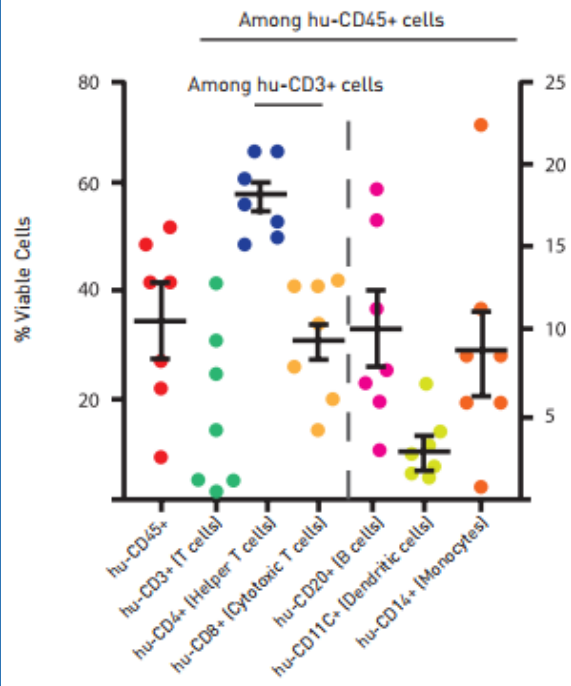


C. Sarcoma

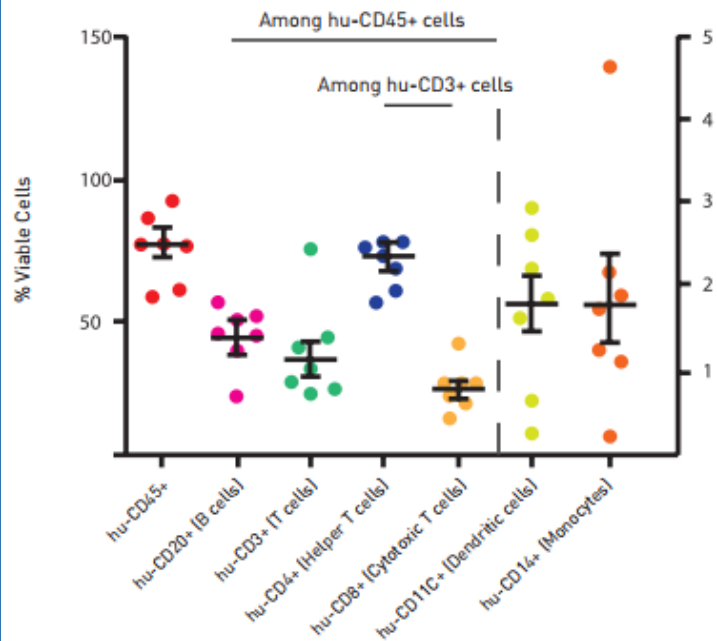




A. Bone Marrow



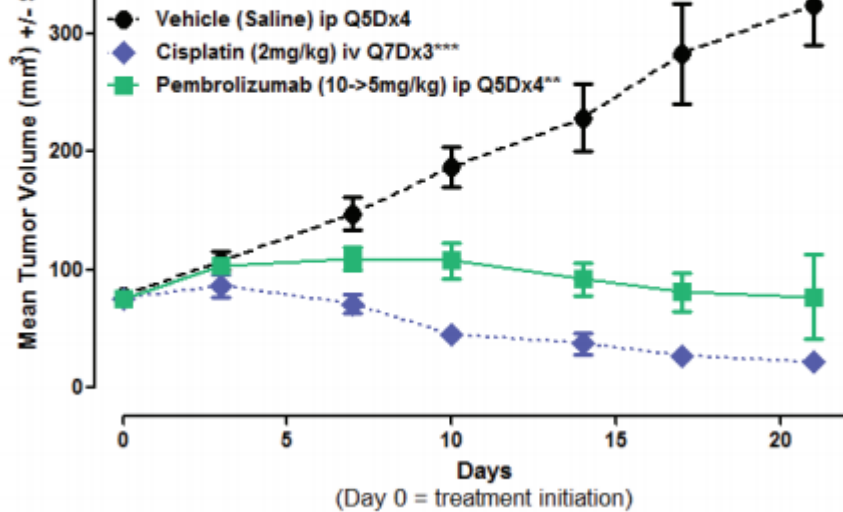
B. Spleen



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

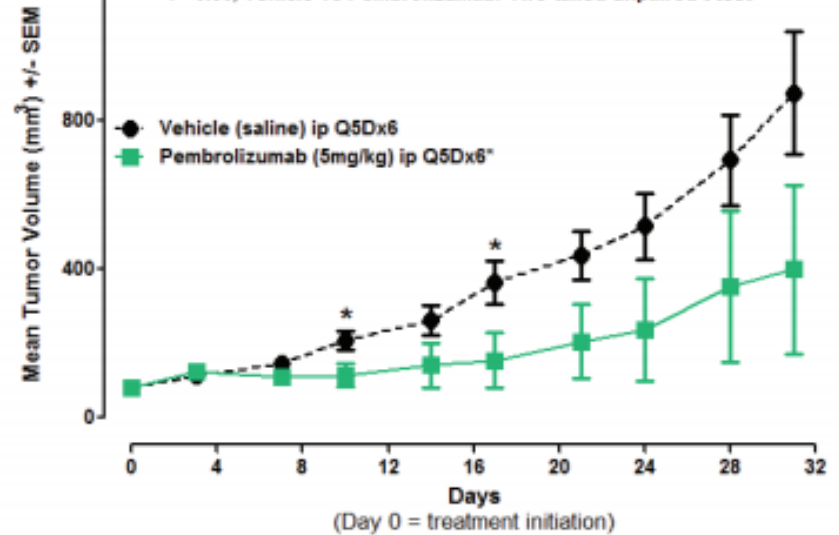
Triple Negative Breast Cancer



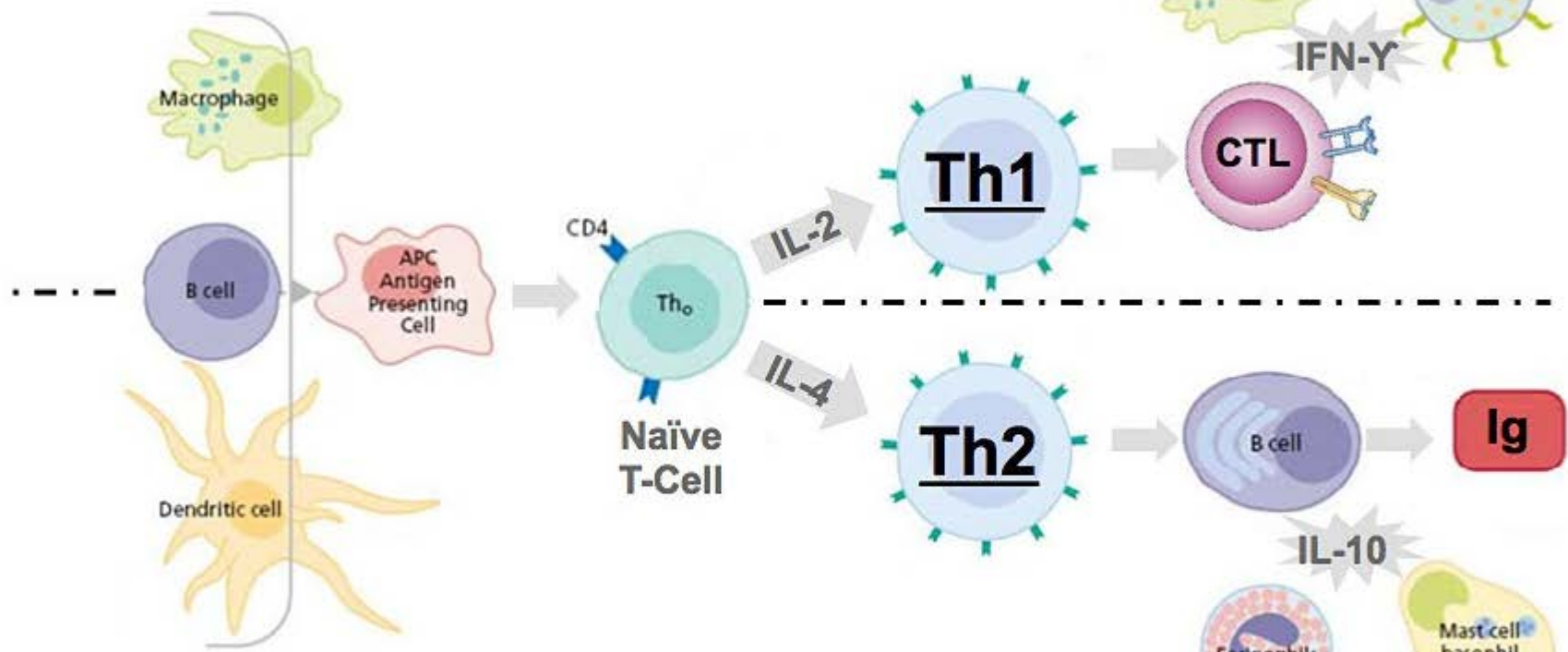
NSCLC

A. Mean Tumor Volume of TM00302 (LG1306P5) PDX in Hu-NSG Mice

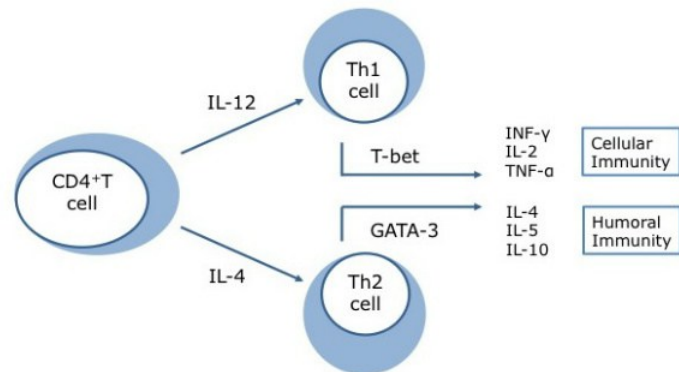
*P<0.05, Vehicle vs Pembrolizumab. Two-tailed unpaired t test



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

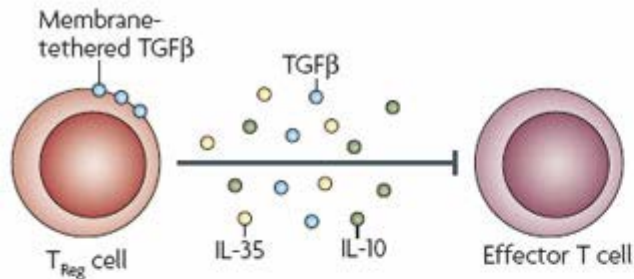


Extracellular pathogen
Humoral immunity (antibodies = Ig)

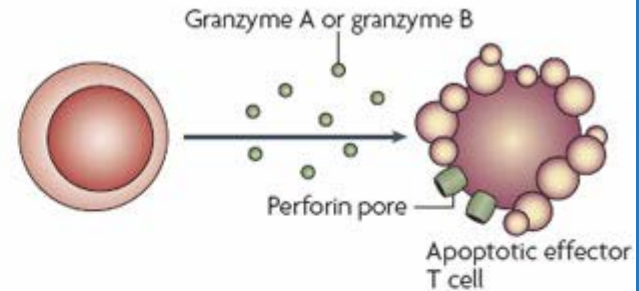


Regulatory T-Cells

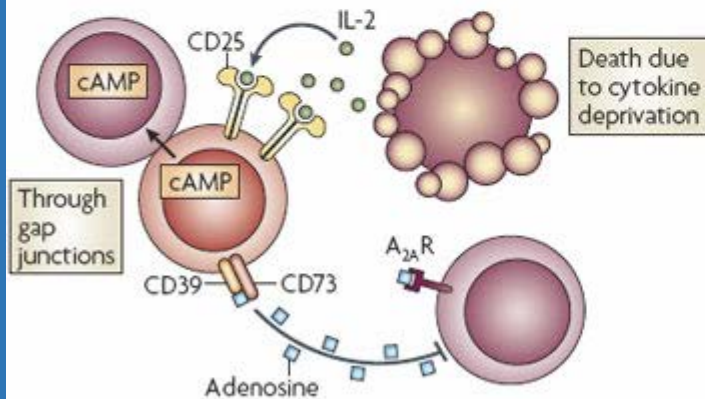
a Inhibitory cytokines



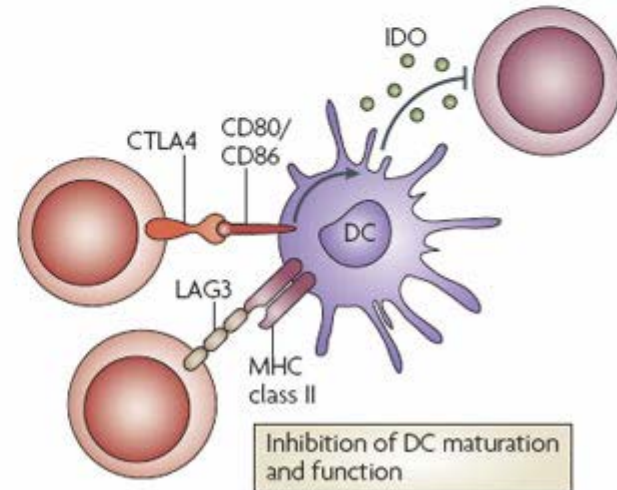
b Cytolysis



c Metabolic disruption



d Targeting dendritic cells



DISTRIBUTION OF Ig ISOTYPES

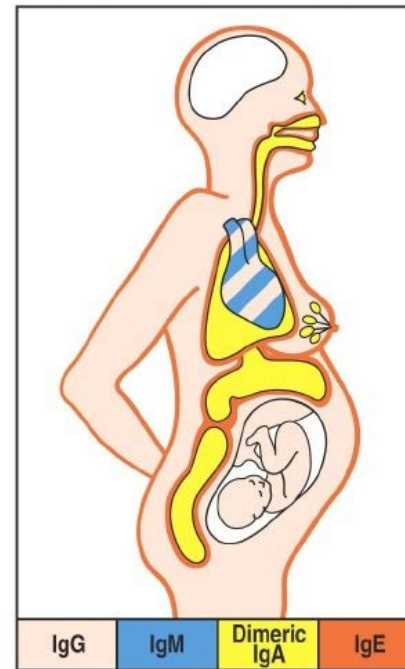



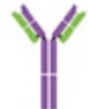

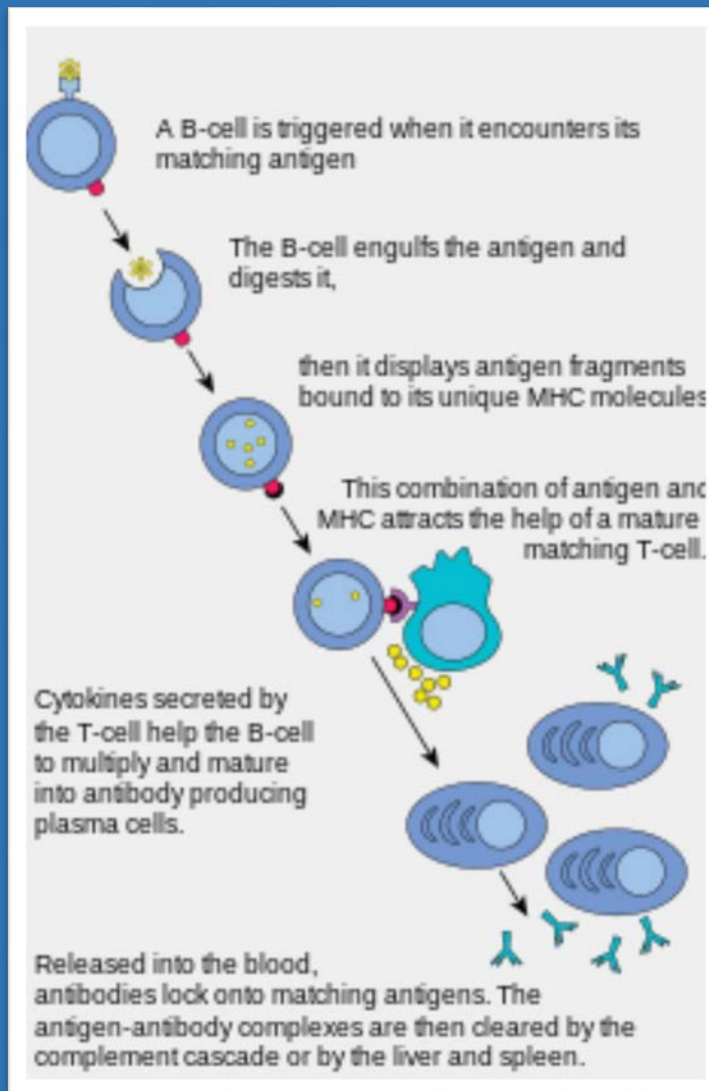


Figure 9-22 Immunobiology, 6/e. (© Garland Science 2005)

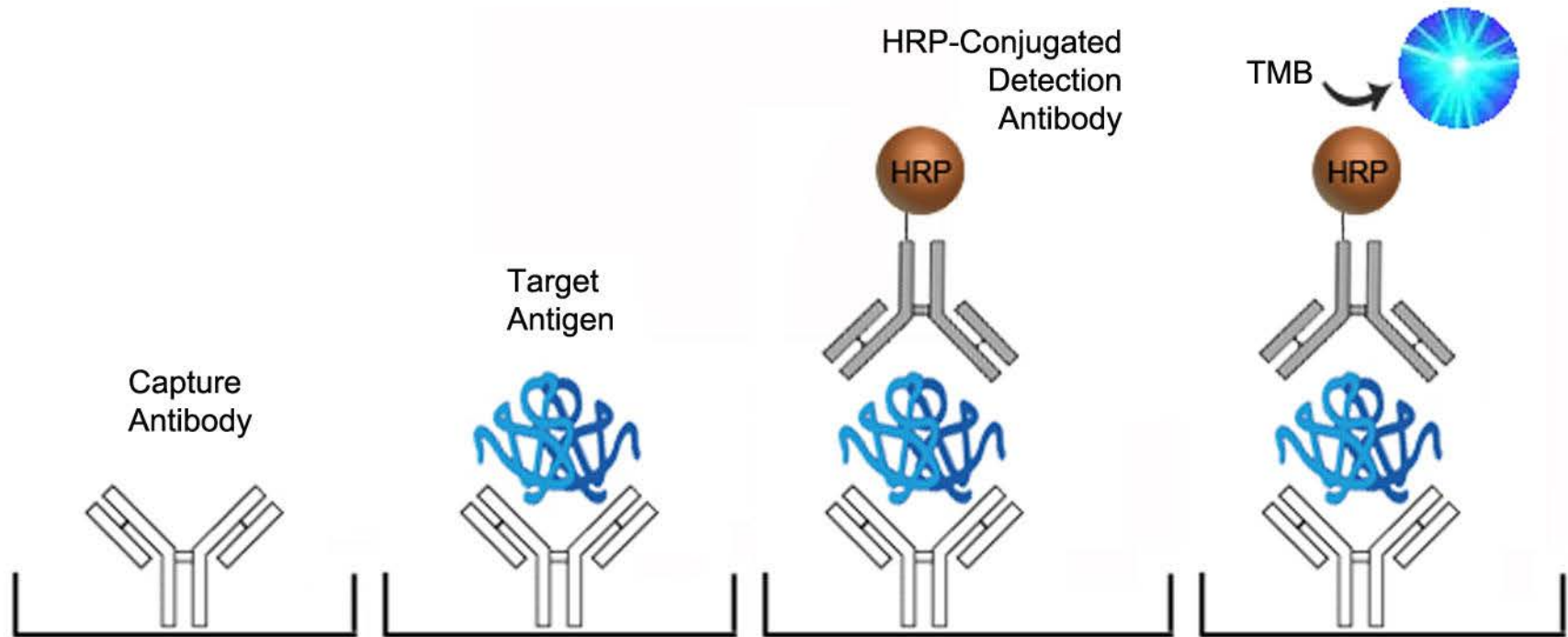
Fig. 9-22

					
	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



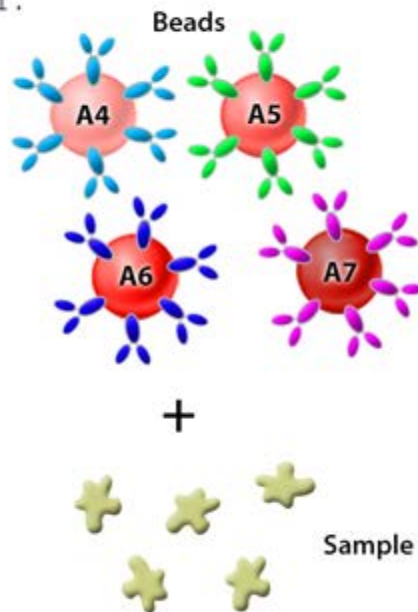
Sandwich ELISA (enzyme-linked immunosorbent assay)



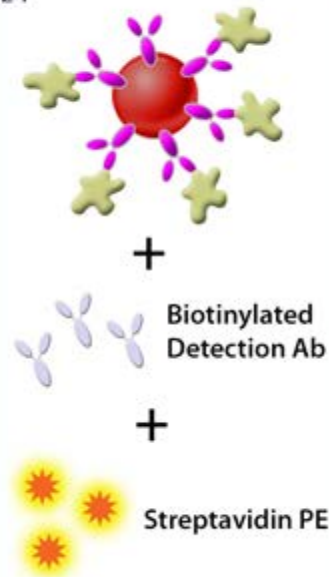
LegendPLEX Cytokine Profiling

PRINCIPLE OF THE ASSAY

1.

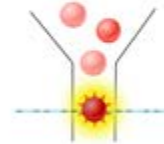


2.



3.

Collect Data by
Flow Cytometry



4.

Quantitate Using LEGENDplex™ Software

