



Today's Menu

Cancer Genomics

Brief review of cancer fundamentals,
What is cancer and what causes it?

Mining Cancer Genomic Data

Hands-on analysis to identify genomic changes in different cancers and identify new targets for therapy

Towards personalized cancer treatments

Recap on how the immune system normally detects cancer cells and how we can predict mutations that can be recognized by T cells

Cancer Immunoinformatics

Hands-on analysis to design personalized cancer vaccines

What is Cancer?

“Cancer is a name given to a collection of related diseases, where some of the body’s cells begin to divide without stopping and spread into surrounding tissue”

Source: <https://www.cancer.gov>

It is estimated that cancer will strike 40% of people at some point in their lifetime with frequently devastating effects.

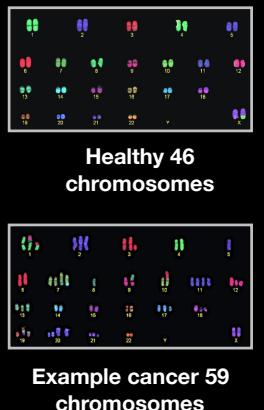
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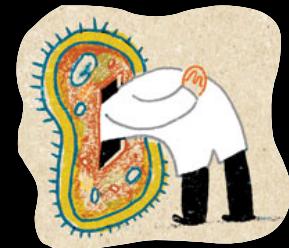
Cancer is a disease of the Genome

- Caused by changes to genes that control the way our cells function, especially how they **grow and divide**.
- A major challenge in treating cancer is that every tumor is different: Each person's cancer has a unique combination of genetic changes (both "driver" & "passenger").
- As the cancer continues to grow, additional changes will occur.

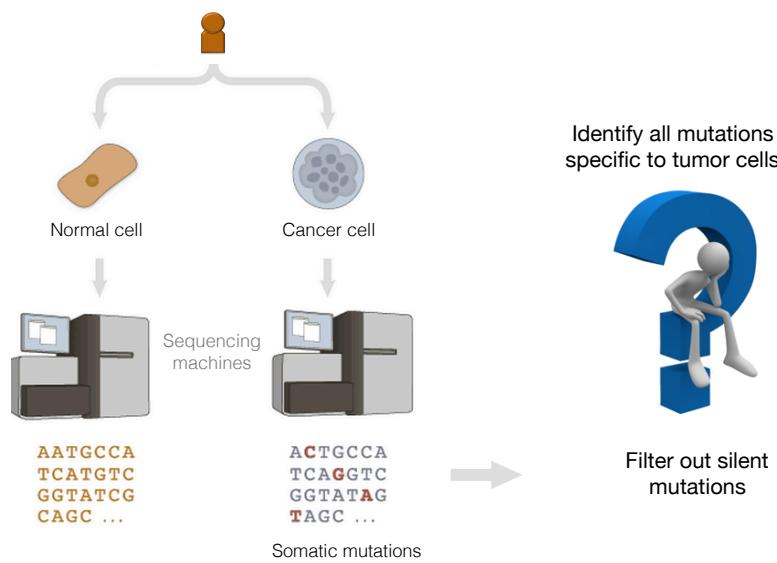


Goals of Cancer Genome Research

- Identify changes in the genomes of tumors that drive cancer progression
- Identify new targets for therapy
- Select drugs based on the genomics of the tumor
- Provide early cancer detection and treatment response monitoring
- Utilize cancer specific mutations to derive neoantigen immunotherapy approaches

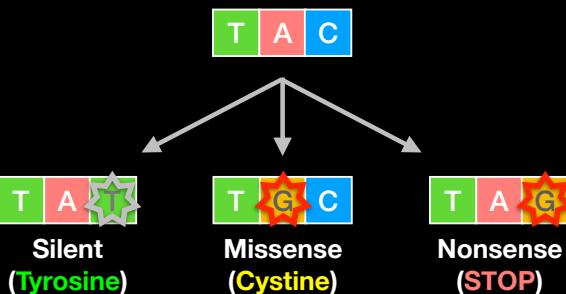


Finding Cancer Associated Mutations



Mutations detected: Point mutations

Original (Tyrosine)



Mutations detected: Indels

Reference Sequence --- C T G G T G A C T A G T T ---

Mutations detected: Indels

Tumor Sequence 1: --- C T G G T G A T T ---

Reference Sequence: --- C T G G T G A **CTAG** T T T ---

Deletion: ↑ CTAG deleted

Mutations detected: Indels

Tumor Sequence 1

Deletion

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

↑ CTAG deleted

Reference Sequence

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

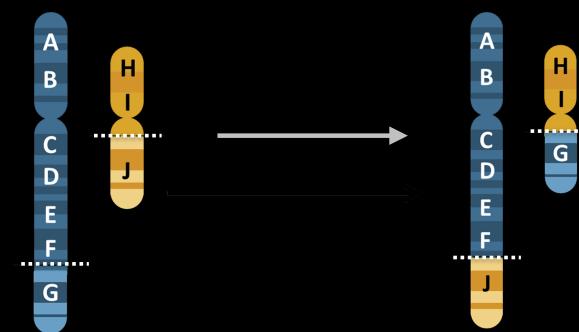
↓ ATCA inserted

Tumor Sequence 2

Insertion

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

Mutations detected: Translocations



(e.g. Philadelphia chromosome (Ph) and found in over 90% of CML patients)

What can go wrong in cancer genomes?

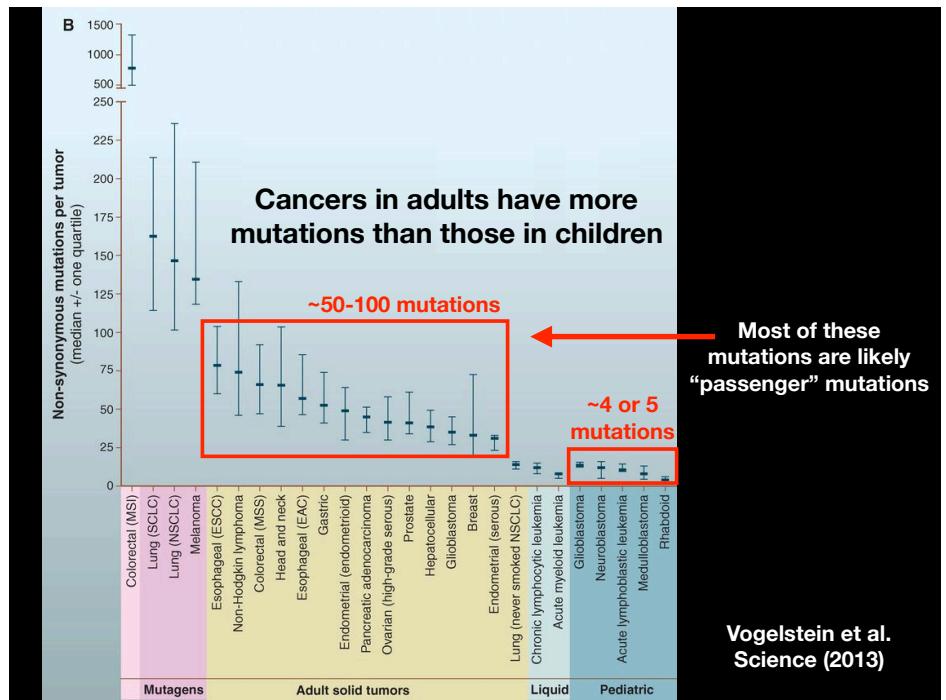
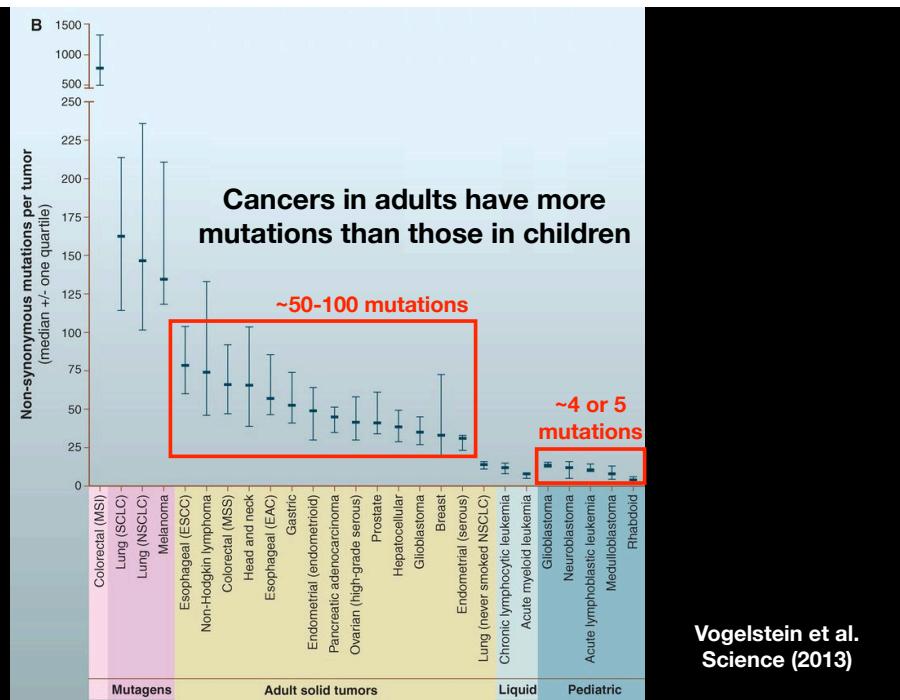
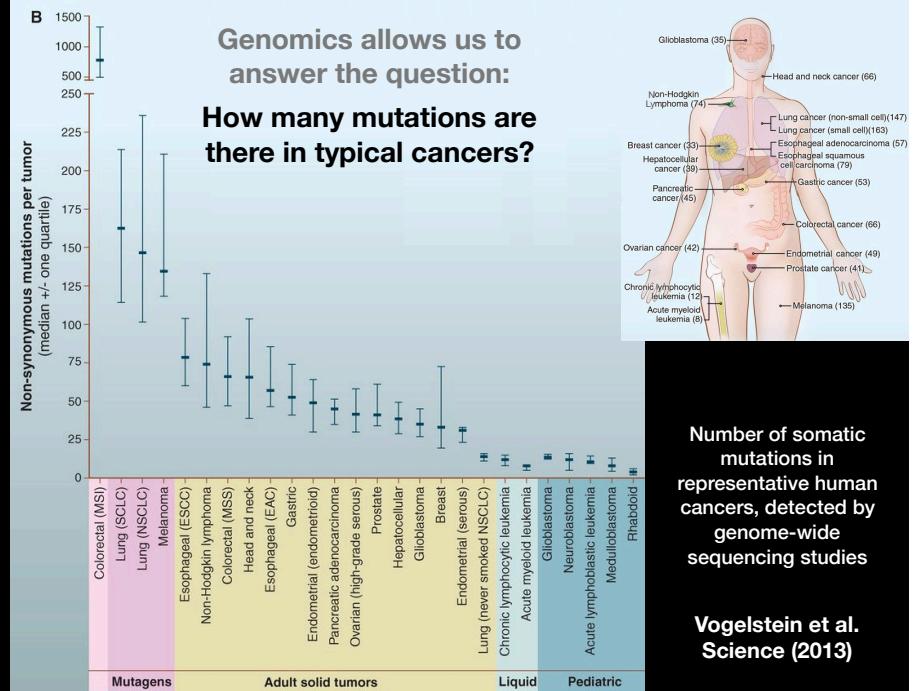
Type of change	Some common technology to study changes
DNA mutations	WGS, WXS
DNA structural variations	WGS
Copy number variation (CNV)	CGH array, SNP array, WGS
DNA methylation	Methylation array, RRBS, WGBS
mRNA expression changes	mRNA expression array, RNA-seq
miRNA expression changes	miRNA expression array, miRNA-seq
Protein expression	Protein arrays, mass spectrometry

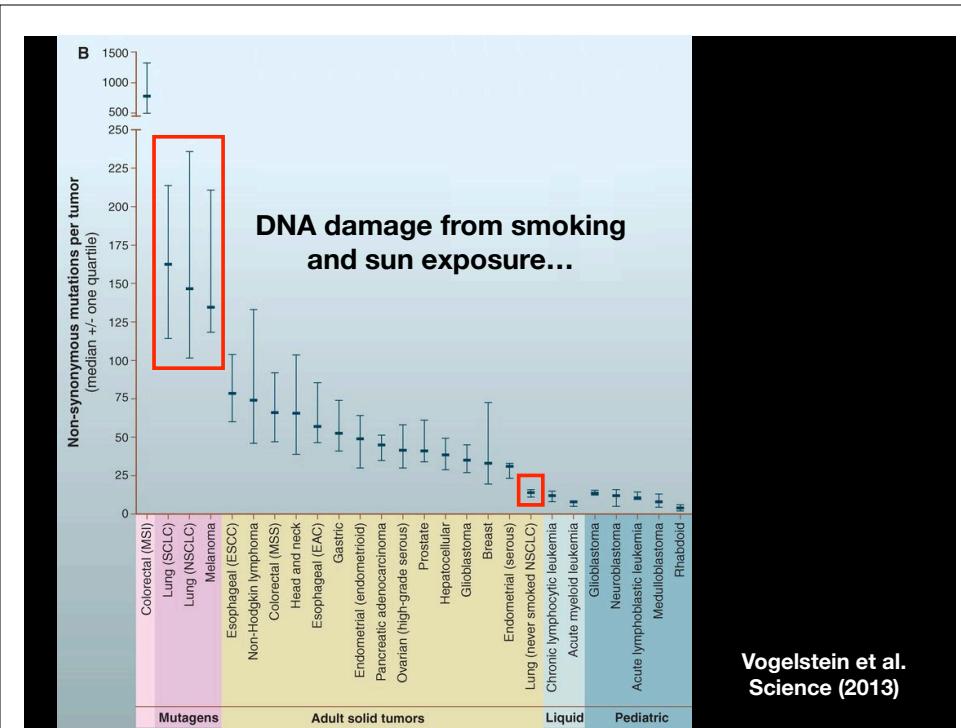
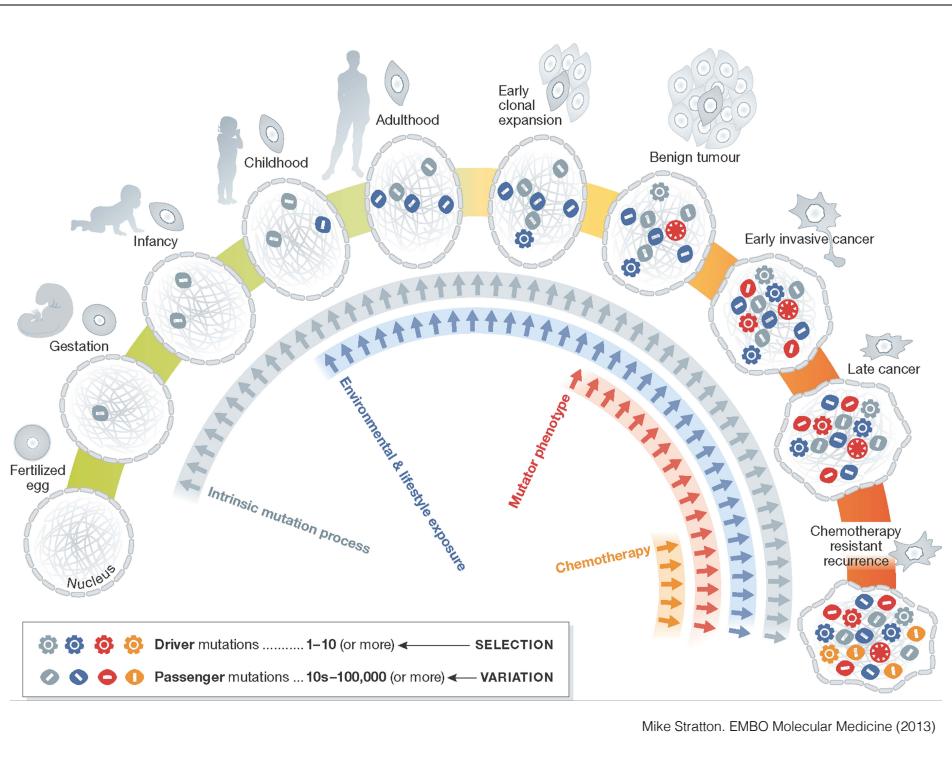
WGS = whole genome sequencing, WXS = whole exome sequencing

RRBS = reduced representation bisulfite sequencing, WGBS = whole genome bisulfite sequencing

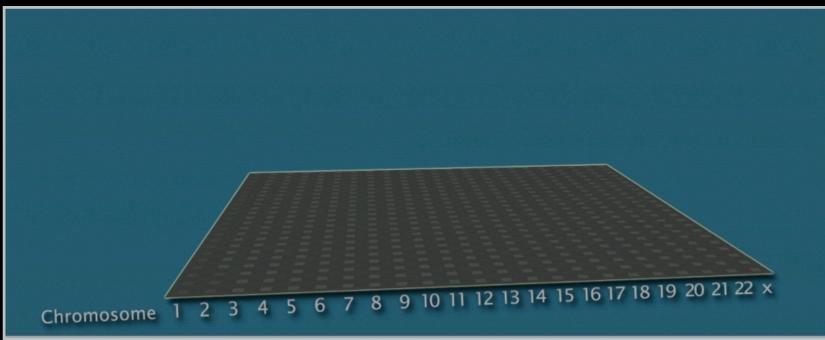
Genomics allows us to answer the question:

How many mutations are there in typical cancers?

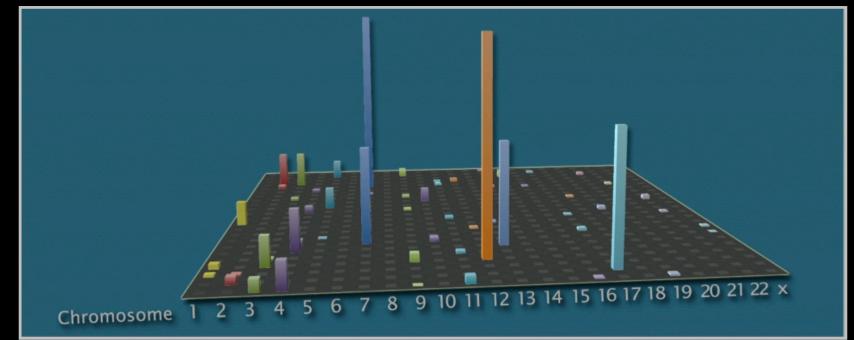




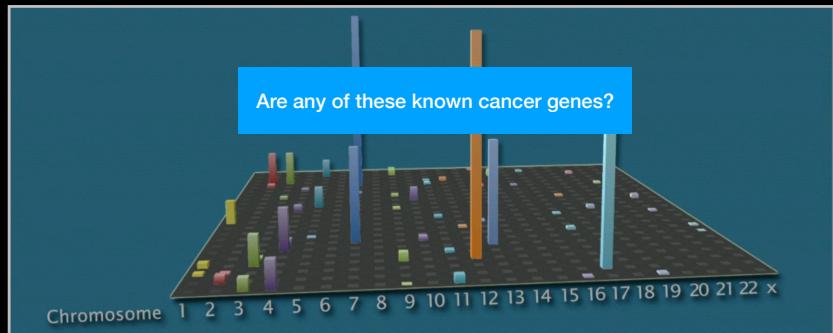
Genomic approaches can identify the genes most commonly mutated in cancer



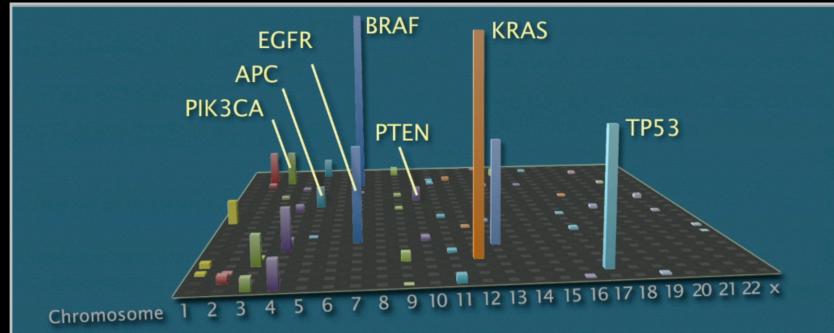
Identifying genes most commonly mutated in cancer



Identifying genes most commonly mutated in cancer



Identifying genes most commonly mutated in cancer

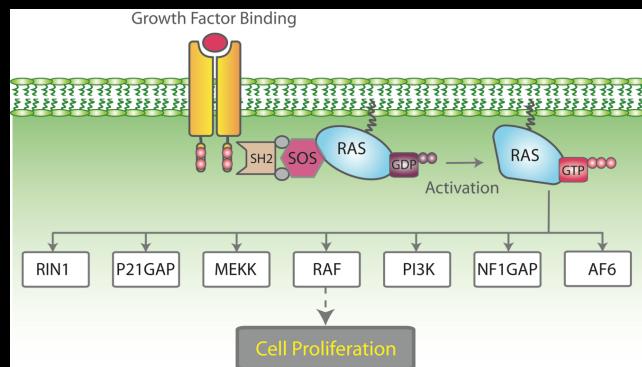


Three Main Types of Cancer Genes:

- **Oncogenes**, such as **Ras**, normally function to accelerate cell division and growth. They can be mutated to act like stuck gas pedals.
- **Tumor suppressor genes**, such as **p53** normal act like breaks. Mutations can cause these breaks to fail.
- **DNA repair genes**, such as **BRCA1 & 2**, normally function to fix minor damage to DNA when it replicates. When these genes are mutated, DNA damage can accumulate and lead to cancer.

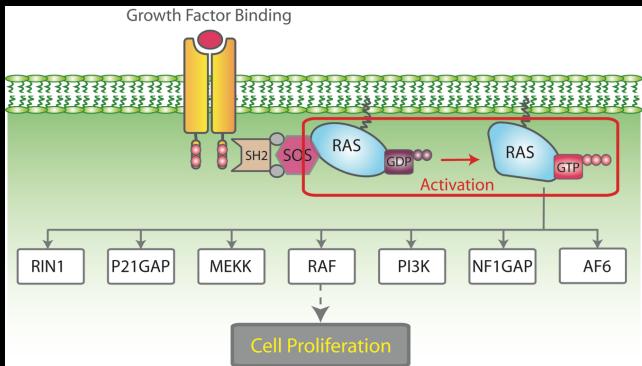
Cell growth and survival genes

Many participate in signaling pathways that promote cell proliferation
(E.G. EGFR, Ras, BRAF, MEK etc.)

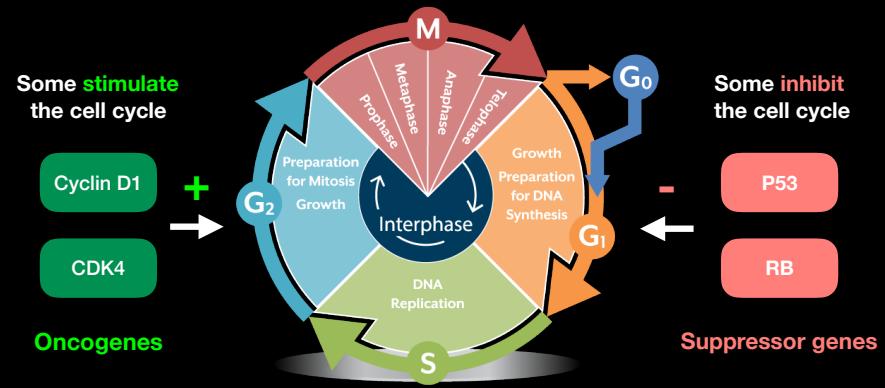


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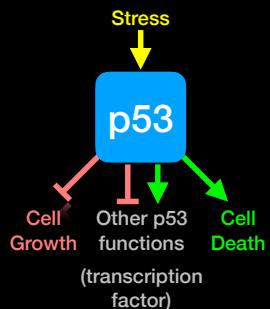
Regulators of Cell Cycle and Cell Death



p53 Regulates Cell Division

Probably the most famous cancer gene that is mutated in about half of all tumors. Often called the '*guardian of the genome*'

- p53 normally shuts down cell division when a cell is stressed (e.g. by DNA damage)
- When DNA is damaged, p53 activates genes that stop cell growth or trigger the cell to die.
- Thus, p53 guards against changes to cells that might lead to tumor formation.
- It appears necessary to inactivate p53 to develop many forms of cancer.

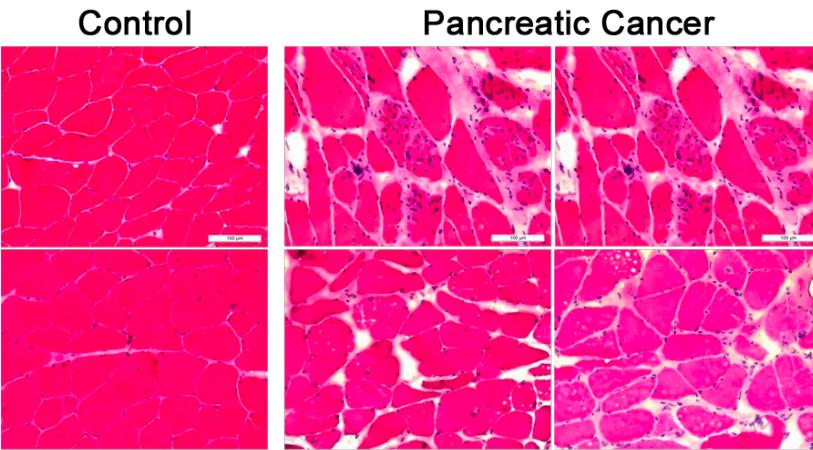


Hands-on time!

https://bioboot.github.io/bgg213_F19/lectures/#18

Part 1 Only Please

Do it Yourself!



Representative H&E micrographs of rectus abdominis biopsies are displayed for two patients without cancer (left) and four patients with pancreatic cancer (right)

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Next Up:

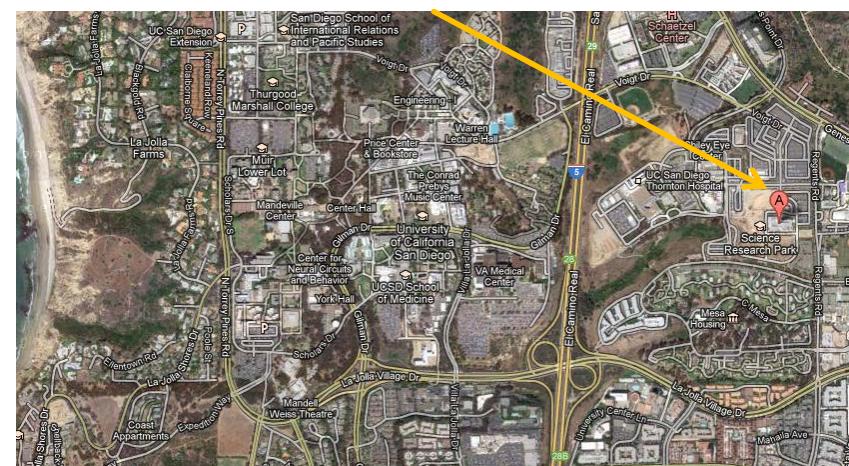
Towards personalized cancer treatments using Immunoinformatics

Bjoern Peters
Zeynep Kosaloglu-Yalcin
La Jolla Institute for Immunology

La Jolla
Institute
FOR IMMUNOLOGY

Life
Without
Disease.®

La Jolla Institute for Immunology (LJI)

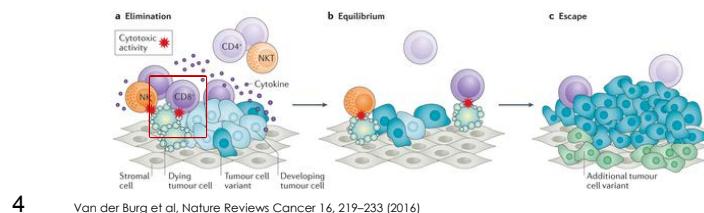


Overview

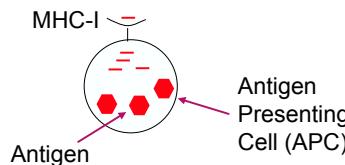
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Cancer immune surveillance and escape

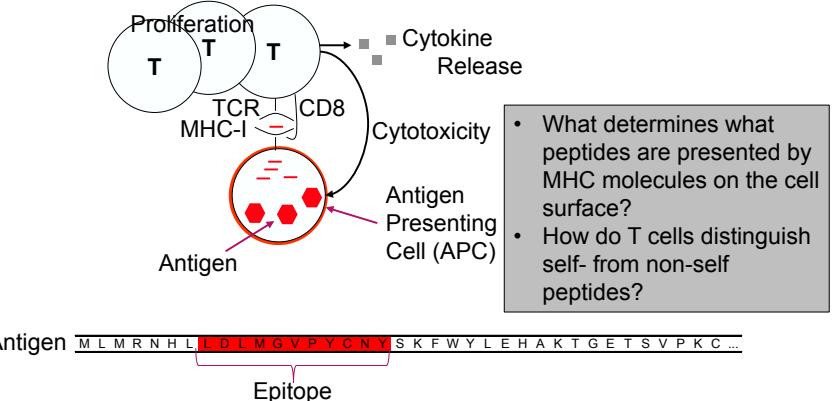
- Mutations in cells occur frequently
- The immune system has the capacity to detect and eliminate such mutated cells, and will do so on a regular basis
- Only when mutated cells find ways to hide from- or suppress an attack by the immune system, they can grow and spread unhindered leading to clinically apparent tumors



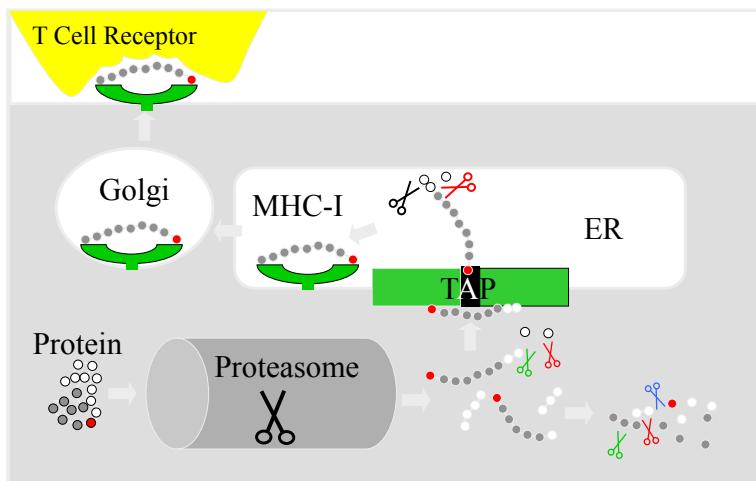
CD8⁺ T cell epitope recognition



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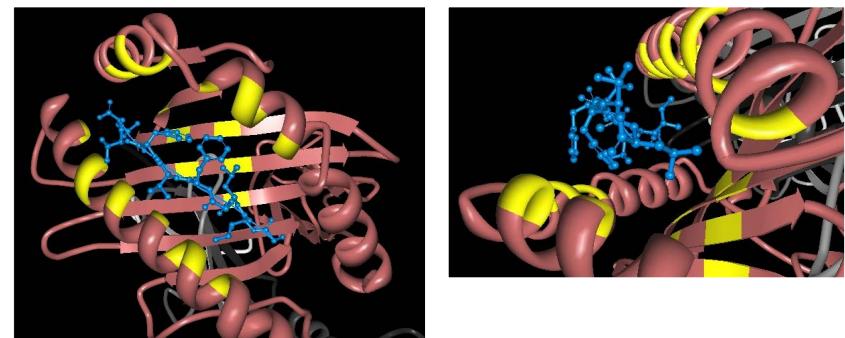


MHC I - Antigen processing and presentation pathway



7 Peters et al, J Mol Biol 2002, Bioinformatics 2003, J Immunol.2003; CMLS 2005 ; Assarson, J Immunol 2007

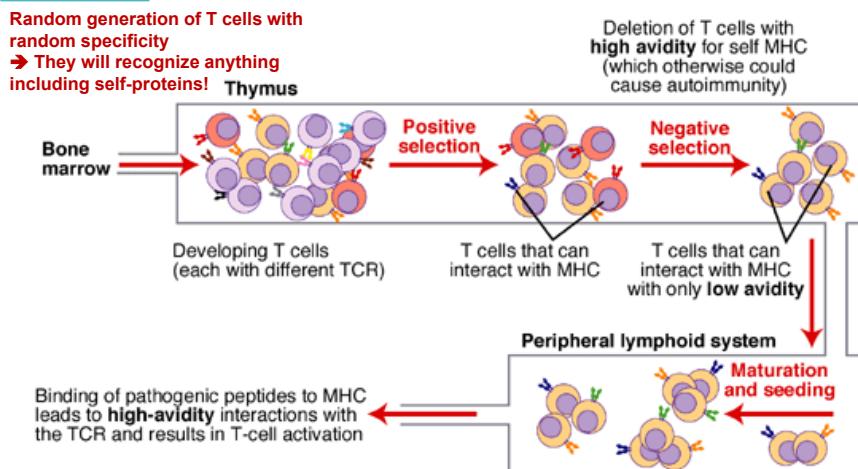
MHC:peptide binding mode



- Each human expresses up to 6 different MHC-I molecules in every cell
- >3000 MHC variants are known
- Distinct binding specificities → individual epitope repertoire

Viewer: Beaver and Ponomarenko, Immunome Research, 2007

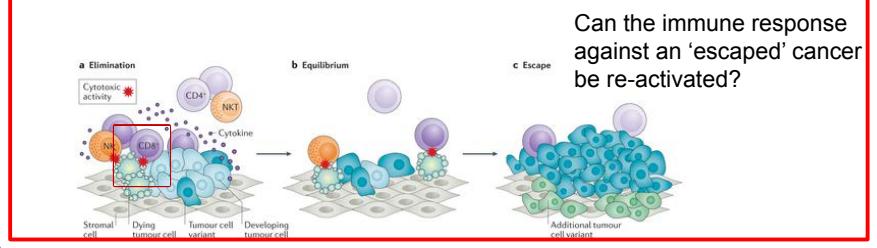
Self-reactive T cells are deleted during maturation



The repertoire of T cells is shaped by both positive and negative selection
Expert Reviews in Molecular Medicine © 1999 Cambridge University Press

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Nobel Prize 2018 in Medicine

- Identification of the molecules PD-1 and CTLA-4 that function as 'T cell brakes' (immune checkpoints)
- Blockade of PD-1 and CTLA-4 results in activation of T cells which has "fundamentally changed the outcome for certain groups of patients with advanced cancer"
- "Similar to other cancer therapies, adverse side effects are seen, which can be serious and even life threatening. They are caused by an overactive immune response leading to autoimmune reactions [...]"



"for their discovery of cancer therapy by inhibition of negative immune regulation"

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<https://www.nobelprize.org/uploads/2018/10/press-medicine2018.pdf>

Rationale for Personalized Cancer Immunotherapy

- Vaccination: Introduce or boost an immune response against a specific target (antigen)
- Cancer cells contain non-self antigens that could be recognized by T cells, but presence of cancer means this mechanism has failed, typically by the tumor suppressing immune responses
- Checkpoint blockade treatments: Block immune suppressive mechanisms to boost T cell immune responses against cancer cells.
- Problem: Checkpoint blockade is unspecific, and will also boost unwanted autoimmune responses
- Personalized Cancer Immunotherapy: Boost anti-tumor response with vaccine containing peptides corresponding to cancer mutations that can be recognized by T cells.

Several trials for personalized cancer vaccines are currently ongoing

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Is the Next Big Step in Cancer Therapy Personalized Vaccines?

UC San Diego Health and La Jolla Institute launch clinical trial harnessing an individual's immune system in a syringe

October 12, 2018 | Yadira Galindo

Ezra Cohen, MD, UC San Diego Health physician scientist, administered the first-of-its-kind personalized cancer vaccine to Tamara Strauss, while Aaron Miller, MD, PhD, UC San Diego Health physician scientist, Tamara's mother, Iris Strauss, and Stephen Schoenberger, PhD, La Jolla Institute for Allergy and Immunology professor of immunology, look on.

neoantigens that generated the strongest T cell response from Tamara's tissue samples. These neoantigens were then presented to Tamara's own T cells and cultured over a two-week period using 50 milliliters of her blood to develop a personalized vaccine.

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Personalized Cancer Immunotherapy



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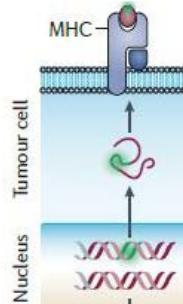
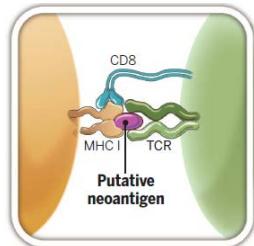
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Neoepitopes (Neoantigens)

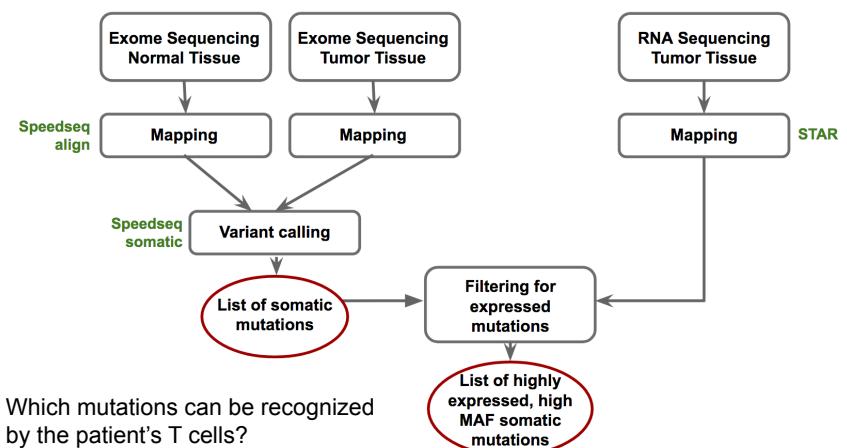
- Cancers genomes accumulate mutations
- Mutations in coding regions are translated in mutated protein sequences
- Mutated peptides can be presented as epitopes on MHC to T cells

Neoepitopes can be recognized by tumor-infiltrating lymphocytes (**TILs**)

Neoepitopes are highly tumor-specific!



DNA and RNA sequencing identifies tumor specific somatic mutations



Which mutations can be recognized by the patient's T cells?
→ Resulting peptides have to bind HLA molecules of the patient

Hands On Part 2.1

- Identify mutated sites in the provided p53 protein sequences
- Identify sequence regions that contain all 9-mer peptides that are only found in the tumor (that contain a mutation)

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Input data from patient:

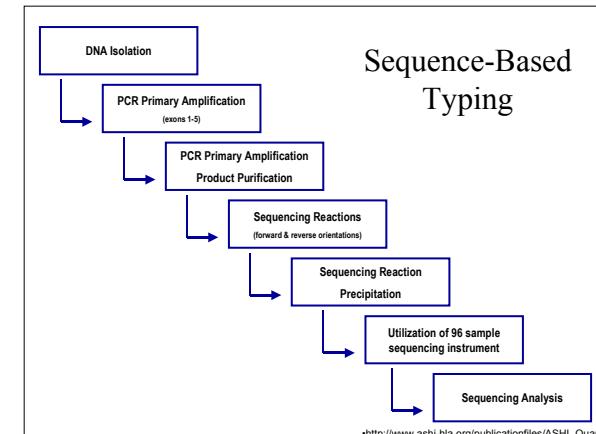
>P53_HUMAN Cellular tumor antigen p53 - Healthy Tissue
MEEPQSDPSVEPPLSQETFSDLWKLLENVNLSPSQAMDDMLSPDDIEQWFTEDPGP
DEAPRMPEAAPVAPAPAAPTAAAPAPAPSWLSSVPSQKTYQGSYGFRLGFLHSGTAK
SVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPGTRVRAMAIIYKQSQHMTEVVRRCPHE
RCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSVVPYEPPEVGSDCTTIHYNYMCNS
SCMGGMNRRPILTTIITLEDSSGNLLGRNSFEVRVCACPGDRRTEENLRKKGEPELPG
PGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEPG
GSRAHSSHLSKKGQSTSRRHKKLMFKTEGPDS

>P53_HUMAN Cellular tumor antigen p53 - Tumor Tissue
MEEPQSDPSVEPPLSQETFSDLWKLLENVNLSPSQAMDDMLSPDDIEQWFTEDPGP
DEAPWMPEAAPVAPAPAAPTAAAPAPAPSWLSSVPSQKTYQGSYGFRLGFLHSGTAK
SVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPGTRVRAMAIIYKQSQHMTEVVRRCPHE
RCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFVHSVVPYEPPEVGSDCTTIHYNYMCNS
SCMGGMNRRPILTTIITLEV

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HLA Typing: Targeted sequencing of HLA locus



*http://www.ashi-hla.org/publicationfiles/ASHI_Quarterly/25_2_2001/highthrustbt3.htm

HLA genotype of patient can be determined in the hospital/lab or can also be determined with immunoinformatics tools using the sequencing data

Measuring and predicting MHC:peptide binding

Experimental Basis: MHC Binding Assay	
Sequence	IC ₅₀
QIVTMFEAL	3.6
LKGDIYKG	308
NFCNLTSAF	50,000
AQSQCRTFR	38,000
CTYAGPFGM	143
CFGNTAVAK	50,000
...	
$\log(\text{IC}_{50}) \sim \text{Binding free Energy}$	
low IC ₅₀ → high affinity	

T cell epitope mapping	ORF 1	ORF 2	ORF 3	ORF 4	ORF 5	ORF 6	ORF 7
	M G Q I V T M F E A L P H I D E V I N I V I	M G L K G P D I Y K G V Y Q F K S V E F D M S H L N L T	M H N F C N L T S A F N K T F D H T L M S I V S S L H L S I D G N S N Y	M S A Q S O C R T F R G R V L D M F R T A F G G K Y M R S G W G W T G S D	M H C T Y A G P F G M S R I L L S Q E K T K F F T R R L A G T F T W T L S	M K C F G N T A V A K C N V N H D A E F C D M L R L I D Y N K A A L S K F	M L M R N H L I L M G V P I C N Y S K F W Y L E H A K T G E T S V P K C

Impossible to measure all peptides

→ Predict binding peptides using machine learning

Find function F_i in F_1, F_2, F_3, \dots
 $F_i(\text{Sequence}) \approx \text{Affinity}$

Many different approaches
(ANN, SVM, HMM, LP, ...)

Calculate scoring matrix from affinities

Machine learning PSSM = Minimize the difference between predicted and measured binding affinities by varying the matrix values

N peptides with measured binding affinities

log (IC ₅₀)	Peptide
0.50	FQPQNGSFI
0.72	ISVANKIYM
2.37	RVYEALYYV
3.42	FQPQSGQFI
3.46	LYEKVKSQL
4.07	FKSVEFDMS
4.18	FQPQNGQFH
4.24	VLMPLPVWFL
4.39	YMTLQGVVF
4.40	EDVKNAVGV
4.90	VFYEQMKRF
...	

HLA A*0201									
1	2	3	4	5	6	7	8	9	0
A -0.3	0.8	-0.3	-0.2	-0.3	0.0	0.0	0.9	0.9	
C 0.2	0.9	0.0	0.3	-0.5	-0.1	0.1	0.2	0.4	
D 0.8	0.9	-0.4	-0.3	0.3	0.2	0.4	0.3	0.6	
E 0.6	-0.4	0.7	-0.2	0.1	-0.4	-0.2	-0.2	-0.5	
F 0.3	0.5	-0.5	0.1	-0.1	0.0	-0.3	-0.4	0.8	
G -0.2	0.1	0.3	-0.1	0.0	0.4	0.3	-0.1	0.2	
H 1.1	0.9	-0.1	0.4	0.1	0.2	0.0	0.2	0.8	
I -0.4	0.7	-0.4	0.1	-0.1	-0.4	-0.5	0.5	1.4	
K -0.3	0.0	1.1	0.1	0.1	0.6	0.9	0.2	0.9	
L 0.0	1.9	-0.4	-0.2	0.0	-0.2	0.0	-0.1	1.1	
M 0.7	-1.2	-0.7	0.2	-0.6	0.0	0.0	0.0	-0.8	
N -0.1	0.3	0.1	0.3	-0.1	-0.3	0.0	0.2	0.7	
P 1.2	0.5	0.6	0.3	0.4	0.0	-0.4	0.5	0.7	
Q 0.4	-1.1	0.0	0.1	0.4	-0.2	-0.3	0.2	0.7	
R -0.2	0.9	1.0	0.3	0.1	0.4	0.7	0.0	0.9	
S 0.3	0.1	0.1	-0.4	0.1	0.3	-0.2	-0.1	0.2	
T -0.2	-0.5	0.1	0.4	0.1	-0.5	0.2	0.0	-0.1	
V -0.1	0.9	-0.1	0.2	0.0	-0.3	0.1	0.1	1.9	
W 0.0	0.7	-0.5	-0.2	-0.1	0.2	-0.3	-0.1	0.4	
Y -0.3	0.2	-0.6	0.2	0.0	0.4	-0.4	-0.3	0.8	

Offset: 4.3

Predictions available as webserver

- Immune Epitope Database (IEDB) Analysis resource
- <http://tools.iedb.org/mhci/>

MHC-I Binding Predictions

Prediction Method Version 2013-02-22 [Older versions]

Specify Sequence(s)

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences. [Browse for sequences in NCBI](#)

Or select file containing sequence(s) Choose File No file chosen

Choose sequence format auto detect format

Choose a Prediction Method IEDB recommended Help on prediction method selections

Specify what to make binding predictions for

MHC source species human

Show only frequently occurring alleles: Select MHC allele(s): Select HLA allele reference set:

Allele Length Upload allele file

Specify Output

Sort peptides by Percentile Rank

Show All predictions

Output format XHTML table

Email address (optional)

Submit Reset

Prediction Method Version 2013-02-22 [Older versions]

Specify Sequence(s)

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences. ([Browse for sequences in NCBI](#))

```
>Region 1
SPLPSQAMLDLMLSPDD
>Region 2
DPGPDEAPWIPPEAAPPV
```

Or select file containing sequence(s) No file chosen

Choose sequence format

Choose a Prediction Method

Prediction Method [Help on prediction method selections](#)

Specify what to make binding predictions for

MHC source species

Show only frequently occurring alleles: [?](#)
Select MHC allele(s)

Select HLA allele reference set: [?](#)

Allele [?](#)

Specify Output

Sort peptides by

Show

Output format

Email address (optional) [?](#)

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Prediction Method Version 2013-02-22 [Older versions]

Specify Sequence(s)

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences. ([Browse for sequences in NCBI](#))

```
>Region 1
SPLPSQAMLDLMLSPDD
>Region 2
DPGPDEAPWIPPEAAPPV
```

Or select file containing sequence(s) No file chosen

Choose sequence format

Choose a Prediction Method

Prediction Method [Help on prediction method selections](#)

Specify what to make binding predictions for

MHC source species

Show only frequently occurring alleles: [?](#)
Select MHC allele(s)

Select HLA allele reference set: [?](#)

Allele [?](#)

HLA-A*01:01 **HLA-A*02:01**
 HLA-A*02:06
 HLA-A*03:01
 HLA-A*11:01
 HLA-A*23:01
 HLA-A*24:02
 HLA-A*25:01
 HLA-A*26:01
 HLA-A*29:02
 HLA-A*30:01
 HLA-A*30:02
 HLA-A*31:01
 HLA-A*32:01

Specify Output

Sort peptides by

Show

Output format

Email address (optional) [?](#)

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Prediction Method Version 2013-02-22 [Older versions]

Specify Sequence(s)

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences. ([Browse for sequences in NCBI](#))

```
>Region 1
SPLPSQAMLDLMLSPDD
>Region 2
DPGPDEAPWIPPEAAPPV
```

Or select file containing sequence(s) No file chosen

Choose sequence format

Choose a Prediction Method

Prediction Method [Help on prediction method selections](#)

Specify what to make binding predictions for

MHC source species

Show only frequently occurring alleles: [?](#)
Select MHC allele(s)

Select HLA allele reference set: [?](#)

Allele [?](#)

HLA-A*02:01 **Sp 8**
 9
 10
 11
 12
 13
 14

Specify Output

Sort peptides by

Show

Output format All lengths

Email address (optional) [?](#)

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Prediction Method Version 2013-02-22 [Older versions]

Specify Sequence(s)

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences. ([Browse for sequences in NCBI](#))

```
>Region 1
SPLPSQAMLDLMLSPDD
>Region 2
DPGPDEAPWIPPEAAPPV
```

Or select file containing sequence(s) No file chosen

Choose sequence format

Choose a Prediction Method

Prediction Method [Help on prediction method selections](#)

Specify what to make binding predictions for

MHC source species

Show only frequently occurring alleles: [?](#)
Select MHC allele(s)

Select HLA allele reference set: [?](#)

Allele [?](#)

HLA-A*02:01 9
 10
 11
 12
 13
 14

Specify Output

Sort peptides by

Show

Output format

Email address (optional) [?](#)

MHC-I Binding Predictions

 Loading... please wait.

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Supported by a contract from the [National Institute of Allergy and Infectious Diseases](#), a component of the National Institutes of Health in the Department of Health and Human Services

MHC-I Binding Prediction Results

Input Sequences

#	Name	Sequence
1	Reg 1	SPLPSQAMLDLMLSPDD
2	Reg 2	DPGPDEAPWIMPEAAPPV

Prediction method: IEDB recommended | Low percentile_rank = good binders

[Download result](#) 

Citations

Check to expand the result:

Allele	#	Start	End	Length	Peptide	Method used	Percentile_rank
HLA-A*02:01	2	9	17	9	WMPEAAPPV	Consensus (ann/complib_sidney2008/smm)	0.4
HLA-A*02:01	1	8	16	9	MLDMLMLSPD	Consensus (ann/complib_sidney2008/smm)	2.9
HLA-A*02:01	1	7	15	9	AMLDLMLSP	Consensus (ann/complib_sidney2008/smm)	4.0
HLA-A*02:01	1	5	13	9	SQAMLDLML	Consensus (ann/complib_sidney2008/smm)	7.7
HLA-A*02:01	1	6	14	9	QAMLDLMLS	Consensus (ann/complib_sidney2008/smm)	26.0
HLA-A*02:01	2	5	13	9	DEAPWIMPEA	Consensus (ann/complib_sidney2008/smm)	32.0
HLA-A*02:01	1	1	9	9	SPLPSQAML	Consensus (ann/complib_sidney2008/smm)	33.0
HLA-A*02:01	1	3	11	9	LPSQAMLDL	Consensus (ann/complib_sidney2008/smm)	39.0
HLA-A*02:01	1	4	12	9	PSQAMLDLM	Consensus (ann/complib_sidney2008/smm)	43.0

Evaluating binding predictions

- Percentile rank < 0.5% = high affinity binder
- Percentile rank 0.5%-1% = intermediate binder
- Percentile rank 1% - 2% = low affinity binder
- Percentile rank 2% - 5% = borderline
- Percentile rank >5% is a non-binder

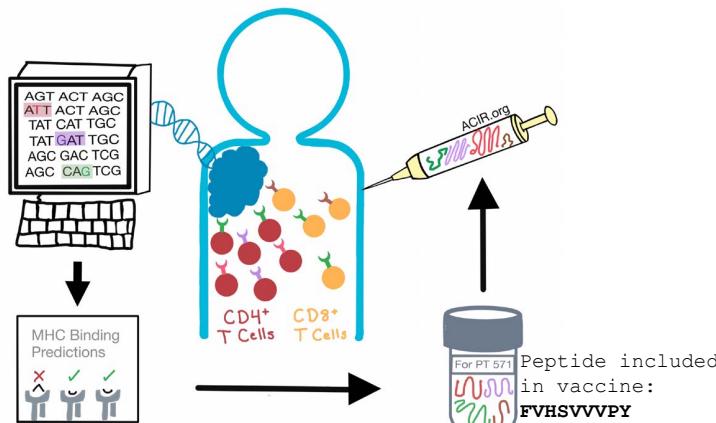
Overview

- Background Biology: The immune system can detect and eliminate mutated cancer cells
- ▶ Background Immunoinformatics: Mutations that can be recognized by T cells can be predicted
- Hands on: Design a personalized cancer vaccine

Hands On Part 2.2

- **Step 1:** Identify sequence regions that contain all 9-mer peptides that are only found in the tumor
- **Step 2:** Run HLA binding prediction to identify 9-mer peptides in the sequence regions unique to the tumor to select peptides that can be presented to T cells in this patient
- **Step 3:** Determine if the identified peptides are specific for the tumor
- Final question: Which peptide would you choose?

Bonus problem: Start with DNA sequencing data



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HLA binding Prediction on IEDB

MHC-I Binding Predictions

Prediction Method Version: 2013-02-22 [Other versions]

Specify Sequence(s)

>D41L
SPLPSQAMLD**L**MLSPDD
>R65W
DPGPDEAP**W**MPEAAPPV
>R213V
YLDDRNTF**V**HHSVVPYE
>D259V
ILTIITLE**V**

Or select file containing sequence(s): Choose File | No file chosen

Choose sequence format: auto detect format

Choose a Prediction Method: IEDB recommended 2.19 + Help on prediction method selections

Show all the method versions: IEDB recommended 2.19 + Help on prediction method selections

Specify what to make binding predictions for: MHC source species: human

Show only frequently occurring alleles: Select MHC allele(s): Select HLA allele reference set:

Allele	Length	Check
HLA-A*02:01	9	<input checked="" type="checkbox"/>
HLA-A*68:01	9	<input checked="" type="checkbox"/>
HLA-B*07:02	9	<input checked="" type="checkbox"/>
HLA-B*35:01	9	<input checked="" type="checkbox"/>

Specify Output

Sort peptides by: Percentile Rank

Show: All predictions

Output format: XHTML table

Email address (optional):

Submit | Re

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Contact & Feedback

Bjoern Peters
bpeters@iji.org

Zeynep Kosaloglu Yalcin
zeynep@iji.org

Feedback link <<https://goo.gl/forms/jfrqJHVq0SzCF4JC3>>

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Bonus Slides (For Reference)

Workflow:

- **Step 1:** Identify sequence regions that contain all **9-mer** peptides that are only found in the tumor
- **Step 2:** Run HLA binding prediction to identify 9-mer peptides in the sequence regions unique to the tumor to select peptides that can be **presented to T cells** in this patient
- **Step 3:** Determine if the identified peptides are specific for the tumor (i.e. are they found anywhere else?)
- **Final question:** Which peptide would you choose?

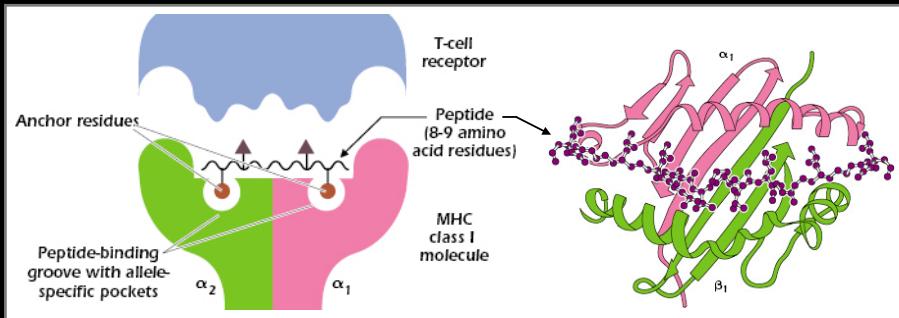
Workflow:

- **Step 1:** Identify sequence regions that contain all **9-mer** peptides that are only found in the tumor
 - ▶ What are the tumor specific amino-acids?
 - ▶ What peptides include these amino acids?
- **Step 2:** Run HLA binding prediction to identify 9-mer peptides in the sequence regions unique to the tumor to select peptides that can be **presented to T cells** in this patient
- **Step 3:** Determine if the identified peptides are specific for the tumor (i.e. are they found anywhere else?)
 - **Which peptide would you choose?**

Workflow:

- **Step 1:** Identify sequence regions that contain all **9-mer** peptides that are only found in the tumor
 - ▶ What are the tumor specific amino-acids?
 - ▶ What peptides include these amino acids?
- **Step 2:** Run HLA binding prediction to identify 9-mer peptides in the sequence regions unique to the tumor to select peptides that can be **presented to T cells** in this patient
- **Step 3:** Determine if the identified peptides are specific for the tumor (i.e. are they found anywhere else?)
 - **Which peptide would you choose?**

Depictions of the peptide bound MHC and T-cell receptor



Note:

- Anchor residues in the peptide bind to the allele-specific pockets of the MHC molecule.
- Certain MHC molecules (alleles) preferentially bind peptides with specific anchor residues in the 8- or 9-amino-acid peptide sequence.
- We want our tumor specific residues to be within 8 to 9-mer sequences bound by a patient HLA alleles!

Reference: <https://oncohemakey.com/how-t-cells-recognize-antigen-the-role-of-the-major-histocompatibility-complex/>

Input data from patient:

>P53_HUMAN Cellular tumor antigen p53 - Healthy Tissue
MEEPQSDPSVEPPLSQETFSDLWKLLENPNVLSPLPSQAMDDMLSPDDIEQWFTEDPGP
DEAPRMPEAAPVAPAPAAPTAAAPAPAPSWLSSVPSQKTYQGSYGFRLGFLHSGTAK
SVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPGTRVRAMAIIYKQSQHMTEVVRRCPHHE
RCSDS DGLAPPQHLIRVEGNLRVEYLDDRNTFRHSV VVPYEPPEVGSDCTTIHYNYMCNS
SCMGGMNRRPILTTIITLEDSSGNLLGRNSFEVRVCACPGDRRTEENLRKKGEPELHP
PGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEPG
GSRAHSSHLSKSKKGQSTS RHKKLMFKTEGP DSD

>P53_HUMAN Cellular tumor antigen p53 - Tumor Tissue
MEEPQSDPSVEPPLSQETFSDLWKLLENPNVLSPLPSQAMDDMLSPDDIEQWFTEDPGP
DEAPWMPEAAPVAPAPAAPTAAAPAPAPSWLSSVPSQKTYQGSYGFRLGFLHSGTAK
SVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPGTRVRAMAIIYKQSQHMTEVVRRCPHHE
RCSDS DGLAPPQHLIRVEGNLRVEYLDDRNTFVHSV VVPYEPPEVGSDCTTIHYNYMCNS
SCMGGMNRRPILTTIITLEV

Tumor Specific Site

*
. . | S P | L | P | S | Q | A | M | L | D | L | M | L | S | P | D | D | K | L | P | Q | . .

Tumor Specific Site

*
. . | S P | L | P | S | Q | A | M | L | D | L | M | L | S | P | D | D | K | L | P | Q | . .
| L | D | L | M | L | S | P | D | D | 1st 9-mer

Tumor Specific Site *

. . . S P L P S Q A M **L** D L M L S P D D K L P Q . . .

1st 9-mer

2nd 9-mer

Tumor Specific Site *

. . . S P L P S Q A M **L** D L M L S P D D K L P Q . . .

1st 9-mer

2nd 9-mer

3rd 9-mer

Tumor Specific Site *

. . . S P L P S Q A M **L** D L M L S P D D K L P Q . . .

1st 9-mer

2nd 9-mer

3rd 9-mer

4th 9-mer

Tumor Specific Site *

. . . S P L P S Q A M **L** D L M L S P D D K L P Q . . .

1st 9-mer

2nd 9-mer

3rd 9-mer

4th 9-mer

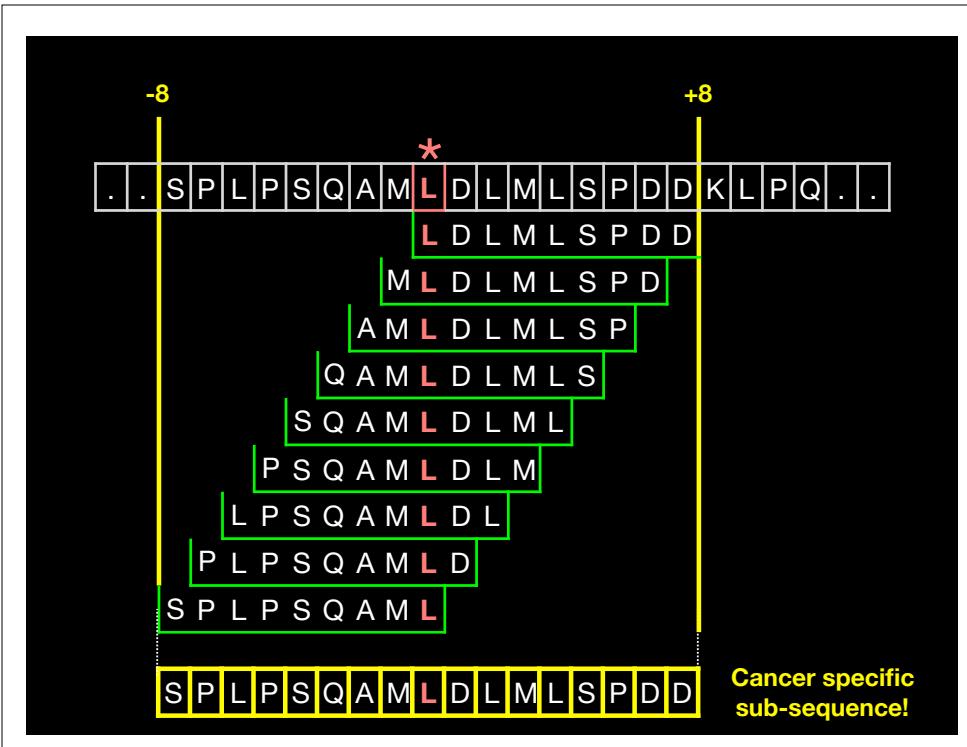
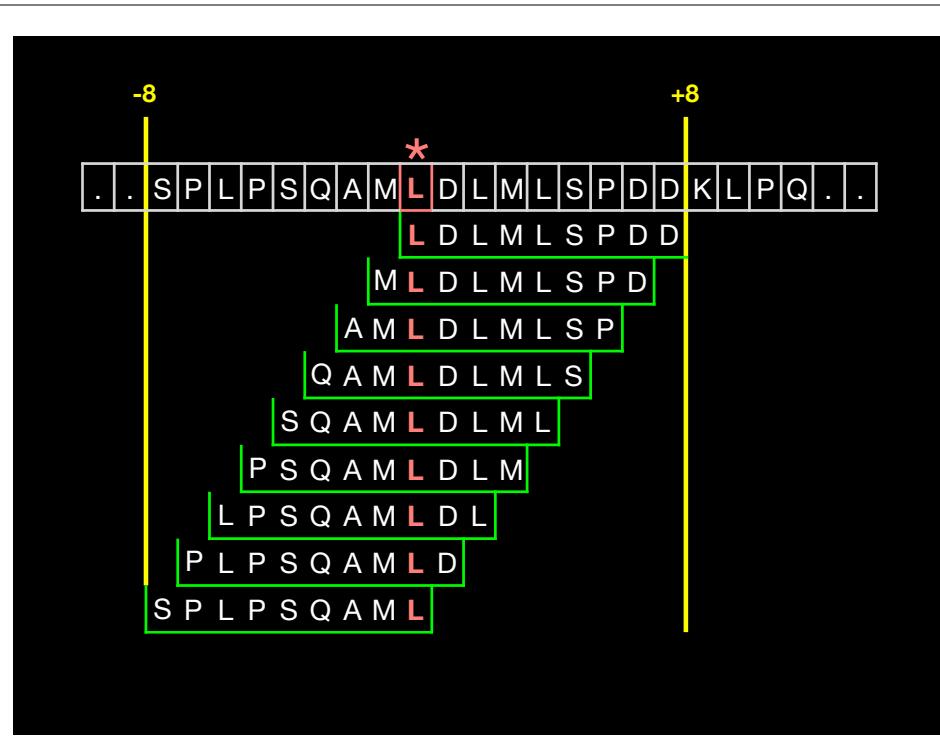
5th 9-mer

6th 9-mer

7th 9-mer

8th 9-mer

9th 9-mer

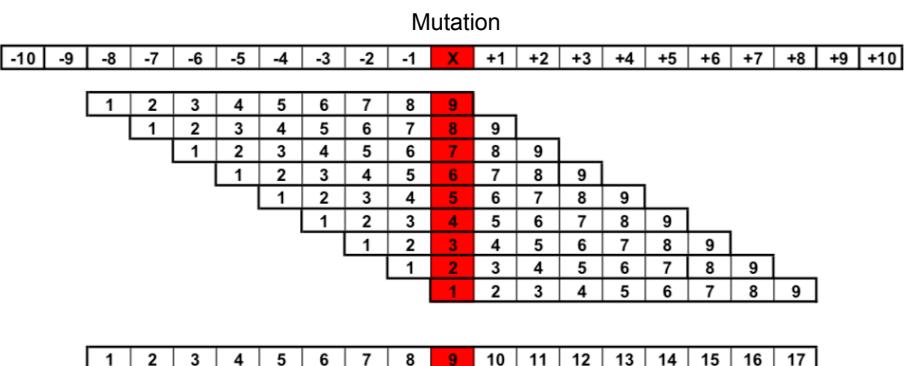


Mutated sites

>P53_HUMAN Cellular tumor antigen p53 - Healthy Tissue
MEEPQSDPSVEPPLSQETFSDLWKLLEPNVLSPLPSQAMDDLMLSPDDEQWFTEDPGP
DEAPRMPPEAPPVAPAPAAPTPAAPAPAPSWPLSSSVPSQKTYQGSYGFRLGFLHSGTAK
SVTCTYSPALNKMFQCQALKTCPQLWVDSTPPGTRVRAMAIIYKQSOHMTTEVVRCPHHE
RCSDSDGLAPPQHLIRVEGNLRVEYLDRNTRHSVVVPYEPPEVGSDCTTIHYNYMCNS
SCMGGMNRRPILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEEENLRKKGEPEHELP
PGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEPG
GSRAHSSHLSKKQGSTSRHKKLMFKTEGPDS

>P53_HUMAN Cellular tumor antigen p53 - Tumor Tissue
MEEPQSDPSVEPPLSQETFSDLWKLLEPNVNLSPSQAMLDLMLSPDDEQWFTEDPGP
DEAPWMPEAAPVAPAPAAPTAAAPAPAPSWPLSSSVPSQKTYQGSYGFRLGLHSGTAK
SVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPGTRVRAMAIFYKQSQHMTEVVRRCPHHE
RCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFVHSVVVPYEPPEVGSDCTTIHYNYMCNS
SCMGGMNRRPILTIITLEV

How long do the sequence regions that contain all 9-mer peptides with the mutation need to be?



→ 17-mer peptide will cover all 9-mers that contain the mutation

Mutated regions

>P53_HUMAN Cellular tumor antigen p53 - Healthy Tissue

```
MEEPQSDPSVEPLSQETFSDLWKLLEPENNVLSPLSQAMDDLMSPDDIEQWFTEDPGP  
DEAPRMPEAAPVAPAPAAPTPAAPAPAPSWSPLSSVPSQKTYQGSYGFRLGFLHSGTAK  
SVTCTYSPALNKMFCQLAKTCPVQLWDSTPPPGTRVRAMAIYKQSQHMTEVVRCPHHE  
RCSDSDGLAPPQHLIRVEGNLRVEYLLDRNTFRHVVVPYEPPEVGSDCCTTIHYNYMCNS  
SCMGGMNRRPILTIIITLEDSSGNLLGRNSFEVRVCACPGRDRTEEENLRKKGEPHHELP  
PGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEPG  
GSRAHSSHILKSKGQSTSRRHKKLMFKTEGPDS
```

>P53_HUMAN Cellular tumor antigen p53 - Tumor Tissue

```
MEEPQSDPSVEPLSQETFSDLWKLLEPENNVLSPLSQAMLDLMLSPDDIEQWFTEDPGP  
DEAPWMPEAAPVAPAPAAPTPAAPAPAPSWSPLSSVPSQKTYQGSYGFRLGFLHSGTAK  
SVTCTYSPALNKMFCQLAKTCPVQLWDSTPPPGTRVRAMAIYKQSQHMTEVVRCPHHE  
RCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFVHSVVVPYEPPEVGSDCCTTIHYNYMCNS  
SCMGGMNRRPILTIIITLEV
```

>D41L

SPLPSQAMLD**I**MLSPPD

>R65W

DPGPDEAP**WMPEAAPV**

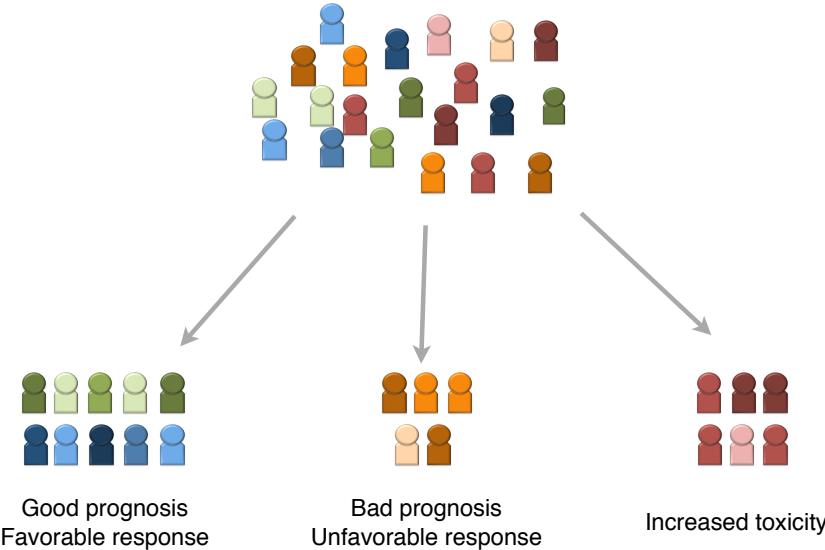
>R213V

YLDDRNTF**V**HSVVVPYE

>D259V

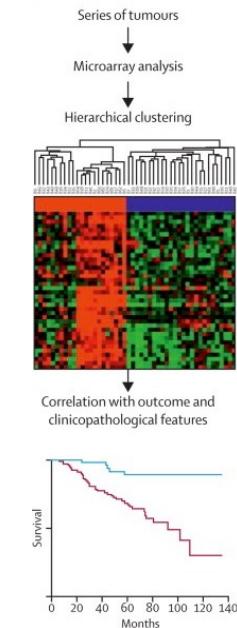
ILTIITLE**V**

Stratify tumors based on molecular patterns

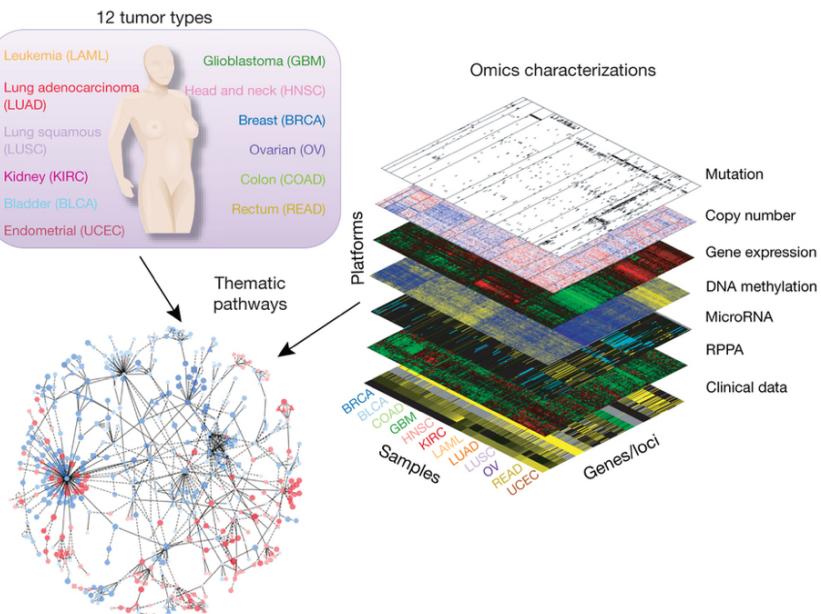


Genetic and genomic approaches can identify a cancer's molecular signature to usefully stratify tumors for treatment

Stratify tumors based on molecular patterns



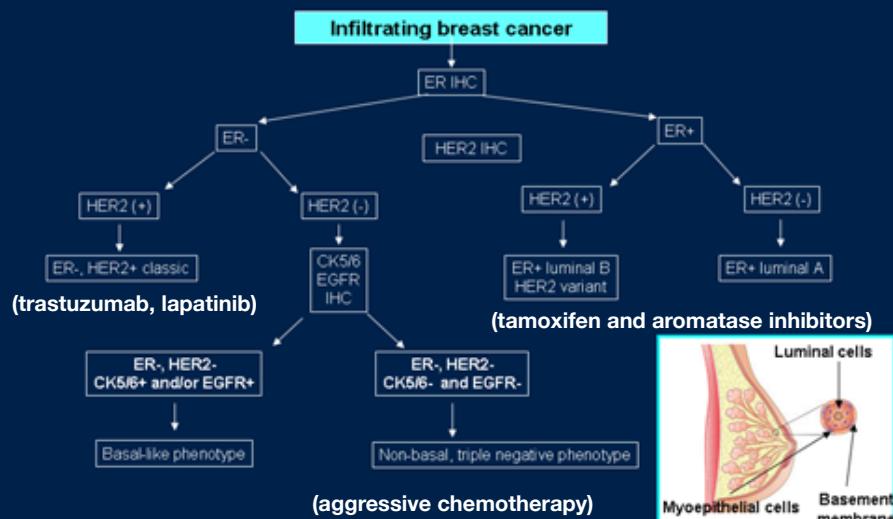
TCGA Pan-Cancer project



For example, breast cancer may be classified into various types based upon which proteins are expressed on the surface of the tumor cells. Breast tumors that express human epidermal growth factor 2 (HER2), estrogen receptor (ER), and progesterone receptor (PR), or are triple negative (do not express HER2, ER, or PR) behave differently and have different prognoses. Tumors that are HER2 positive are treated with medications that bind to HER2 (e.g. trastuzumab, lapatinib) and inhibit its activity. ER and PR are hormone receptors, and ER/PR positive tumors are treated with anti-hormonal therapies (e.g. tamoxifen and aromatase inhibitors). Triple negative tumors have the poorest prognosis and are unlikely to respond to HER2-targeted therapies or anti-hormonal therapies. Such cancers are usually treated very aggressively with chemotherapy.

As more has been learned about the molecular signature of various cancer subtypes, therapies that are specifically targeted to those signatures have been developed. Conventional chemotherapy acts on all rapidly dividing cells and does not distinguish between cancer cells and normal cells.

Classification of Breast Cancer



Readings to find out more...

Leading Edge Review

The Genetic Basis for Cancer Treatment Decisions

Janet E. Dancey,^{1,2} Philippe L. Bedard,^{3,4} Nicole Onetto,¹ and Thomas J. Hudson^{1,5,6,*}

¹Ontario Institute for Cancer Research, Toronto, ON M5G 0A3, Canada

²NCIC-Clinical Trials Group, Queen's University, Kingston, ON K7L 3N6, Canada

³Princess Margaret Hospital, Division of Medical Oncology and Hematology, University Health Network

⁴Department of Medicine

⁵Department of Medical Biophysics

⁶Department of Molecular Genetics

University of Toronto, Toronto, ON M5S 1A1, Canada

*Correspondence: tom.hudson@oicr.on.ca

DOI 10.1016/j.cell.2012.01.014

Personalized cancer medicine is based on increased knowledge of the cancer mutation repertoire and availability of agents that target altered genes or pathways. Given advances in cancer genetics, technology, and therapeutics development, the timing is right to develop a clinical trial and research framework to move future clinical decisions from heuristic to evidence-based decisions. Although the challenges of integrating genomic testing into cancer treatment decision making are wide-ranging and complex, there is a scientific and ethical imperative to realize the benefits of personalized cancer medicine, given the overwhelming burden of cancer and the unprecedented opportunities for advancements in outcomes for patients.

Your Turn

Read and share your thoughts on the following class *Readings*

- Calling cancer's bluff with neoantigen vaccines
- Can genomics help detect early cancer and monitor treatment effectiveness?
- The increasing cost of cancer therapies

https://bioboot.github.io/bimm194_W18/readings/