The Good and The Bad of Human Leukocyte Antigen (HLA)

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Disclosures

- I have no financial relationships with commercial interests to disclose.
- My presentation does not include discussion of off-label or investigational use.

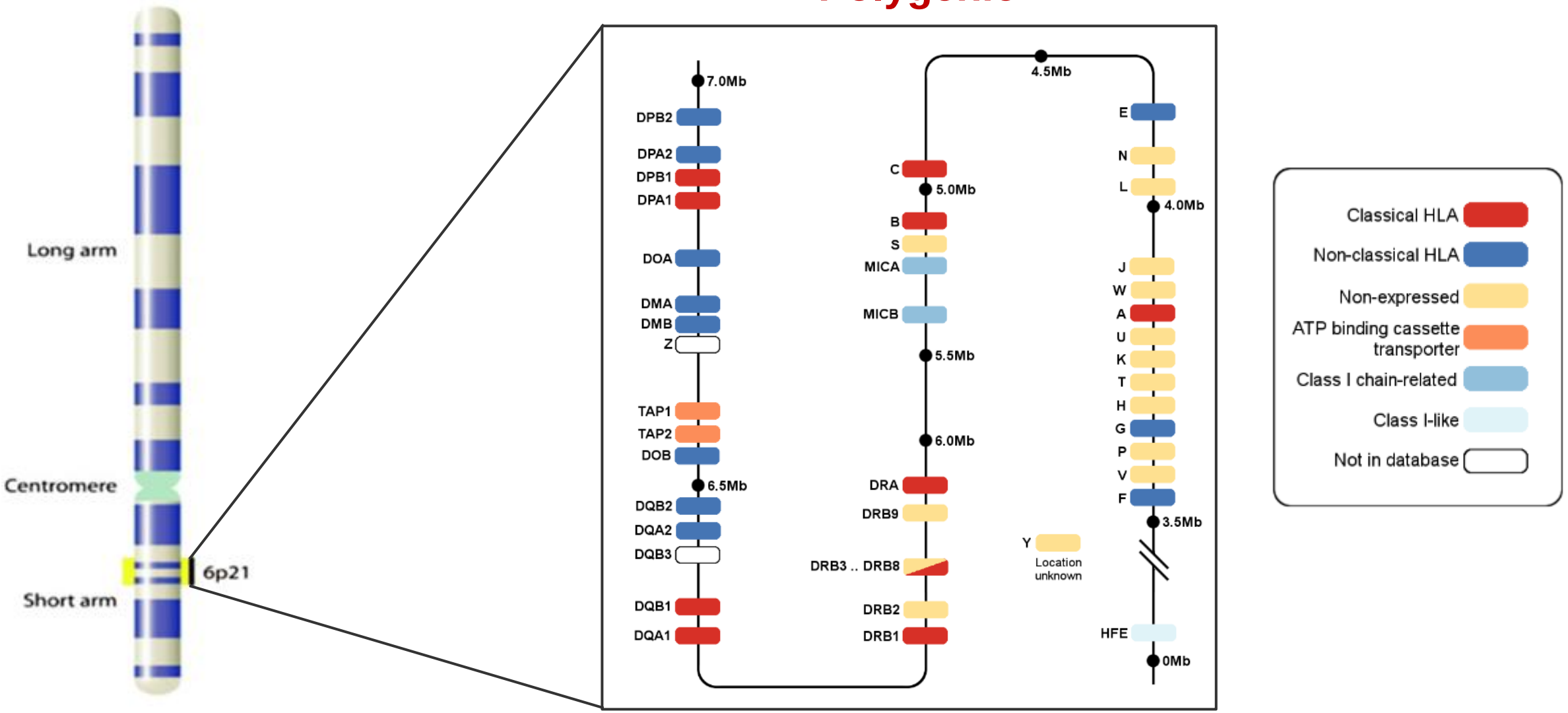
The Good and The Bad of Human Leukocyte Antigen (HLA)

Learning Objectives:

- Define the characteristics of Human leukocyte antigen (HLA).
- Describe the association of HLA alleles with adverse drug reactions (ADR).
- Discuss HLA testing as a companion diagnostic tool in immunotherapy.

Human Leukocyte Antigen (HLA)

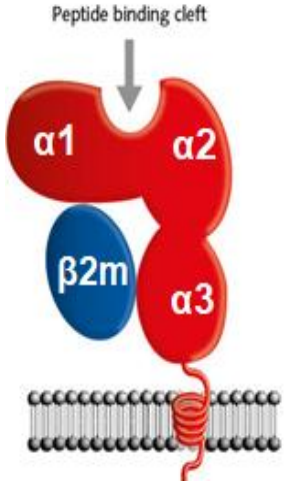
Polygenic



http://hla.alleles.org/

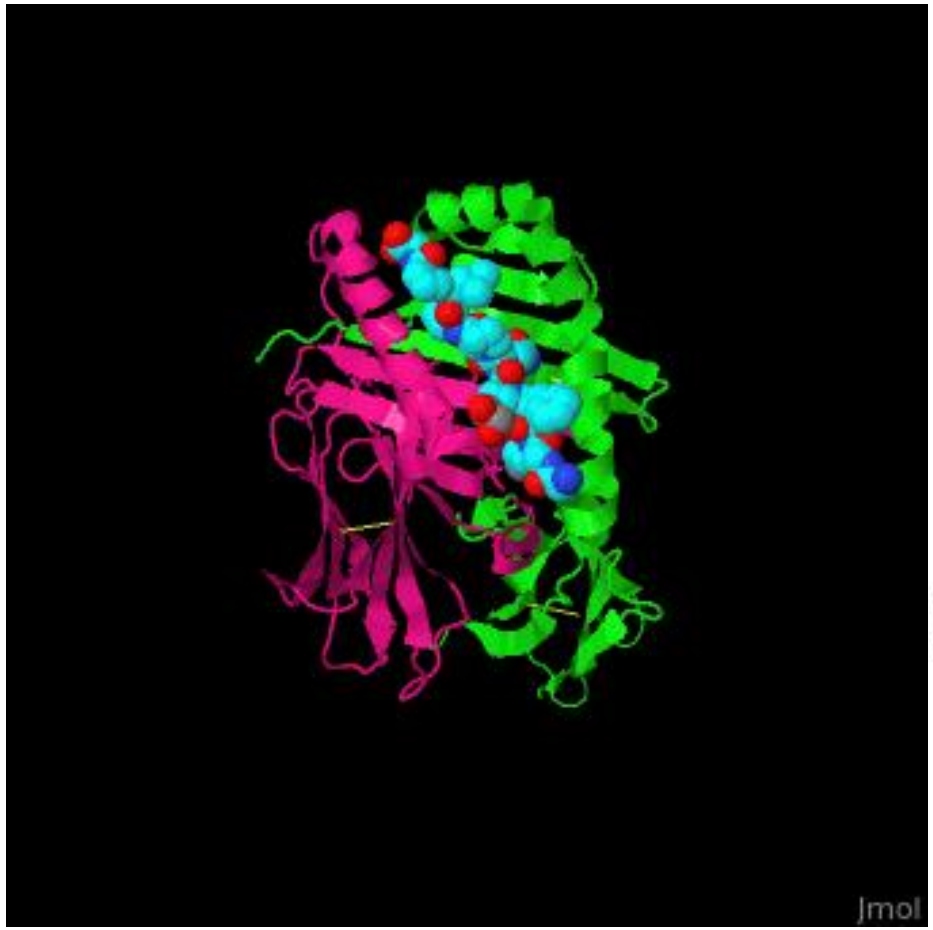
Structure of HLA Proteins

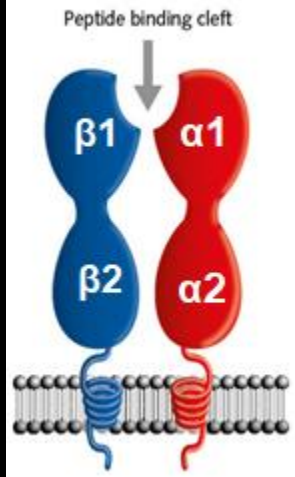
Class I



(HLA – A*02:03) α-chain, β 2-macroglobulin, & peptide

Class II



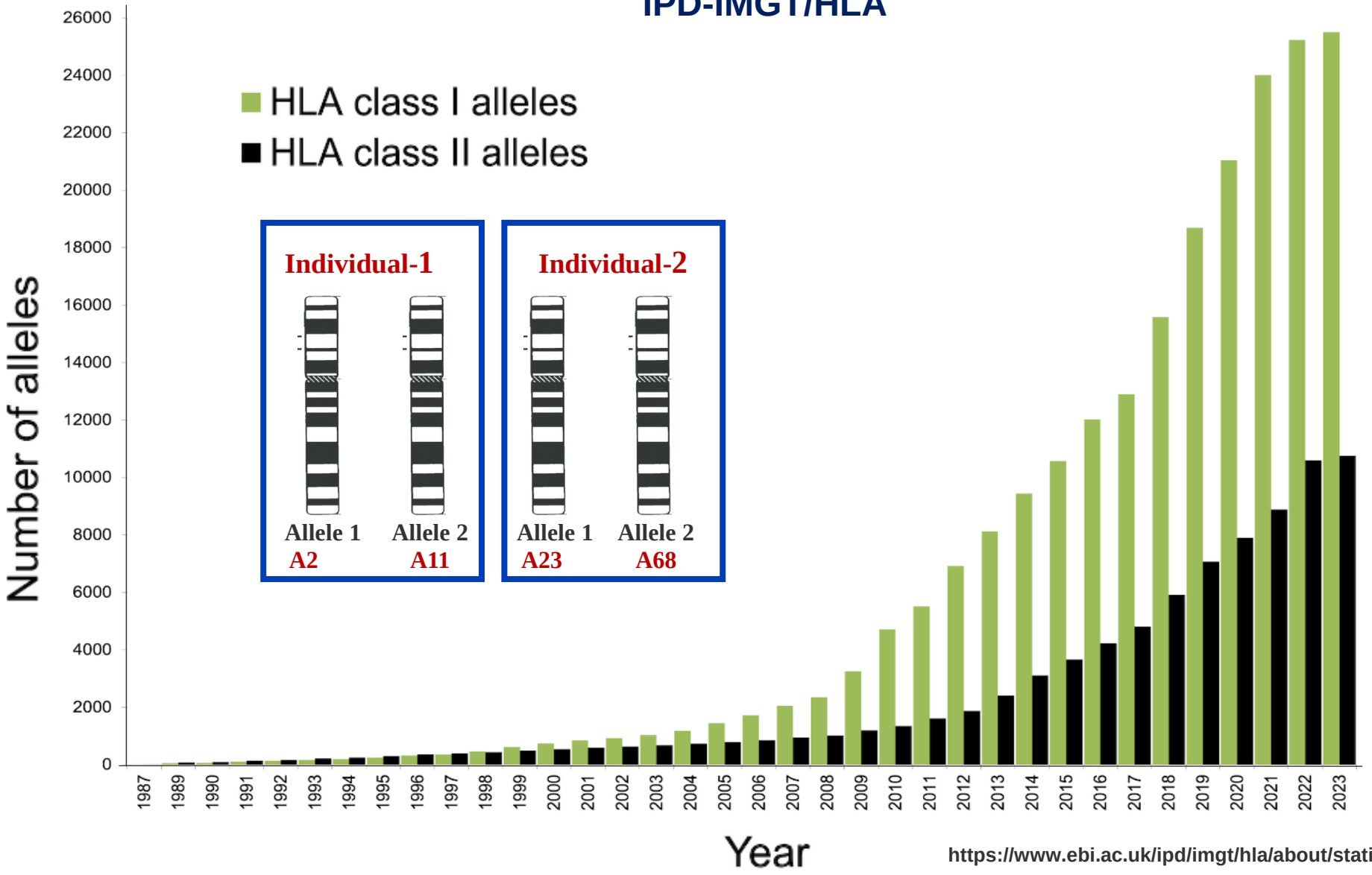


(HLA – DR1) α chain, β chain, and peptide (cyan)

https://proteopedia.org/cgi-bin/pubready

HLA Polymorphism

IPD-IMGT/HLA



https://www.ebi.ac.uk/ipd/imgt/hla/about/statistics/growth/

HLA genes are highly polymorphic

25509 Class I and 10754 Class II alleles

HLA Class I

Gene	A	B	C	E	F	G
Alleles	7,793	9,274	7,761	347	59	117
Proteins	4,548	5,580	4,311	140	11	38
Nulls	404	325	244	10	0	6

HLA Class II

Gene	*DRA*	*DRB*	*DQA1*	*DQA2*	*DQB1*	*DPA1*	*DPA2*	*DPB1*	*DPB2*	*DMA*	*DMB*	*DOA*	*DOB*
Alleles	46	4,419	585	42	2,439	42	558	5	2,332	6	58	71	92
Proteins	5	2,903	281	11	1,501	9	261	0	1,367	0	9	9	14
Nulls	0	192	14	0	109	1	25	0	121	0	0	0	1

HLA Class II-DRB Alleles

Gene	*DRB1*	*DRB2*	*DRB3*	*DRB4*	*DRB5*	*DRB6*	*DRB7*	*DRB8*	*DRB9*
Gene	3,516	1	462	236	192	3	2	1	6
Alleles	2,262	0	345	151	145	0	0	0	0
Proteins	119	0	23	26	24	0	0	0	0

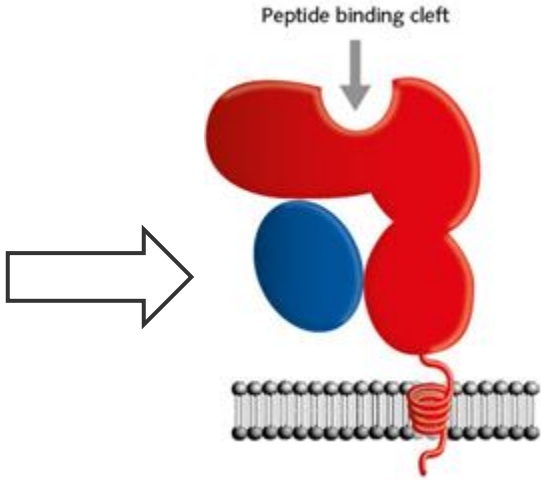
Modified from http://hla.alleles.org/ (Data accessed on 06/12/2023)

HLA Nomenclature

Class I

HLA-A*24:02:01:02L

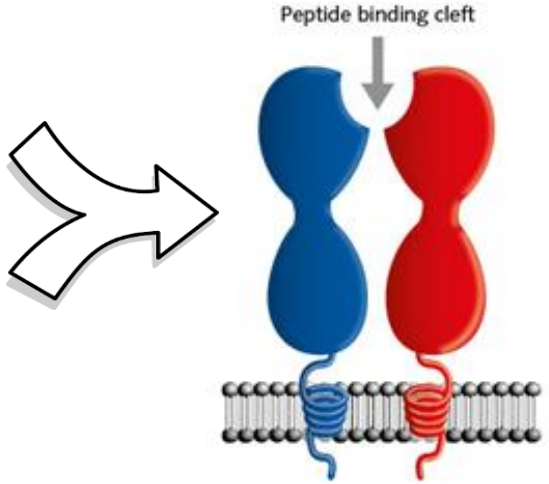
- Gene: HLA-A
- Allele group: 24
- Specific protein: 02
- Synonymous Substitution (coding region): 01
- Substitution in non-coding region: 02
- Protein Expression: L



Class II

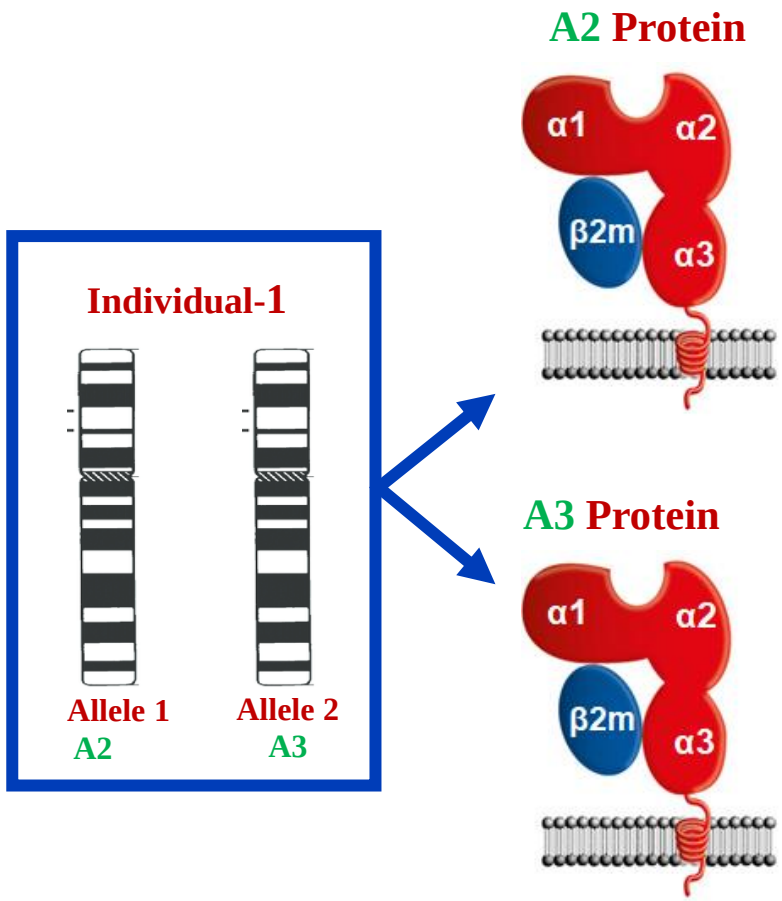
HLA-DQA1*02:01:01:02

HLA-DQB1*04:02:01:01



Additional Characteristics

Co-dominance

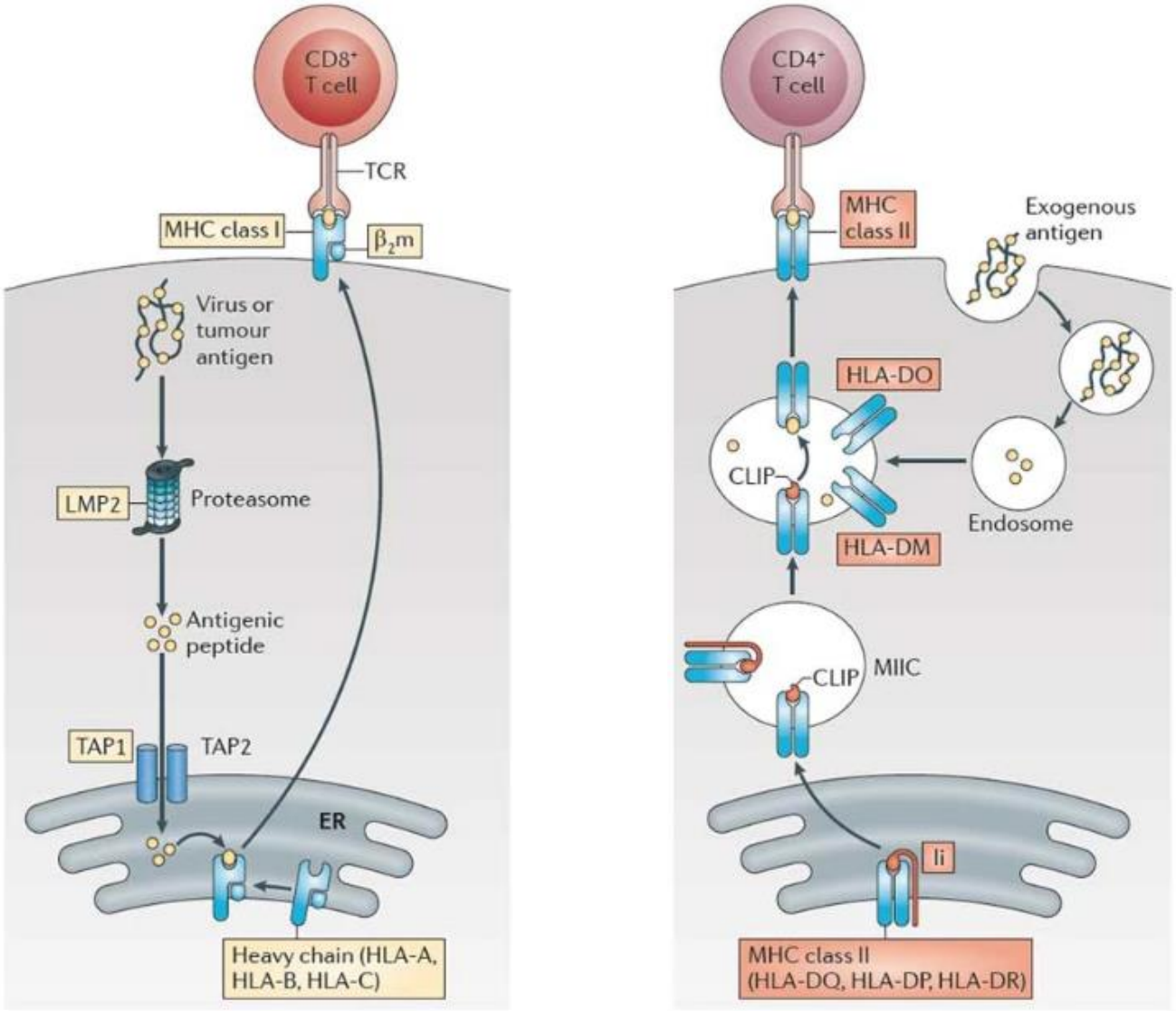


Linkage Disequilibrium

A	C	B	DRB3-4-5	DRB1	DQB1
A*01:01g	C*07:01g	B*08:01g	DRB3*01:01	DRB1*03:01	DQB1*02:01g
A*03:01g	C*07:02g	B*07:02g	DRB5*01:01	DRB1*15:01	DQB1*06:02
A*02:01g	C*07:02g	B*07:02g	DRB5*01:01	DRB1*15:01	DQB1*06:02
A*02:01g	C*05:01g	B*44:02g	DRB4*01:01g	DRB1*04:01	DQB1*03:01g
A*29:02g	C*16:01	B*44:03	DRB4*01:01g	DRB1*07:01	DQB1*02:01g

Function of HLA

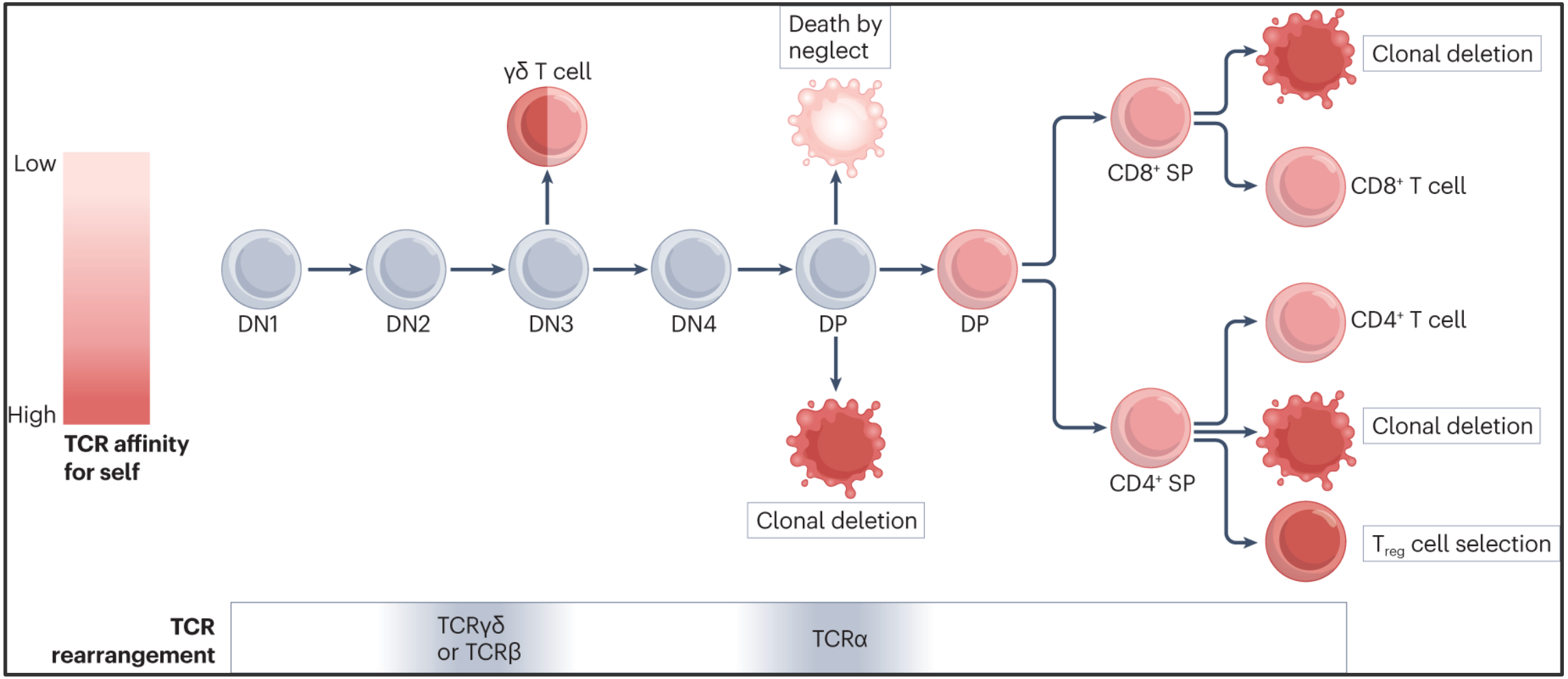
Regulation of Immune response via antigen presentations



Kobayashi, K. et al. Nat Rev Immunol 12, 813–820 (2012).

The Good of HLA

- Thymic selection of T cells
- Discrimination of self and non-self;
- Central tolerance

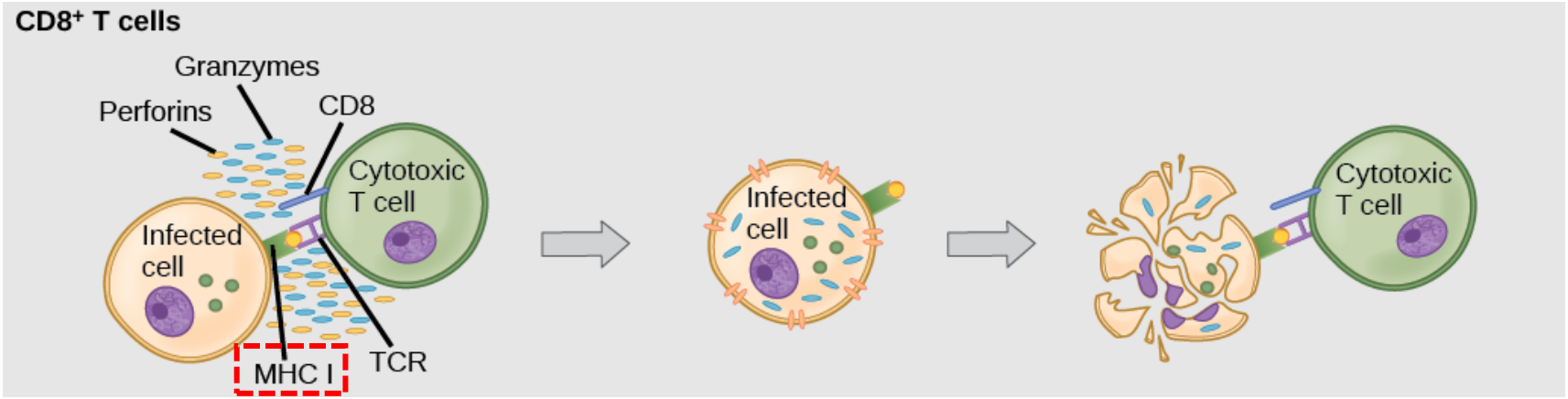


Ashby, K.M., ET. AL. A guide to thymic selection of T cells. Nat Rev Immunol (2023).

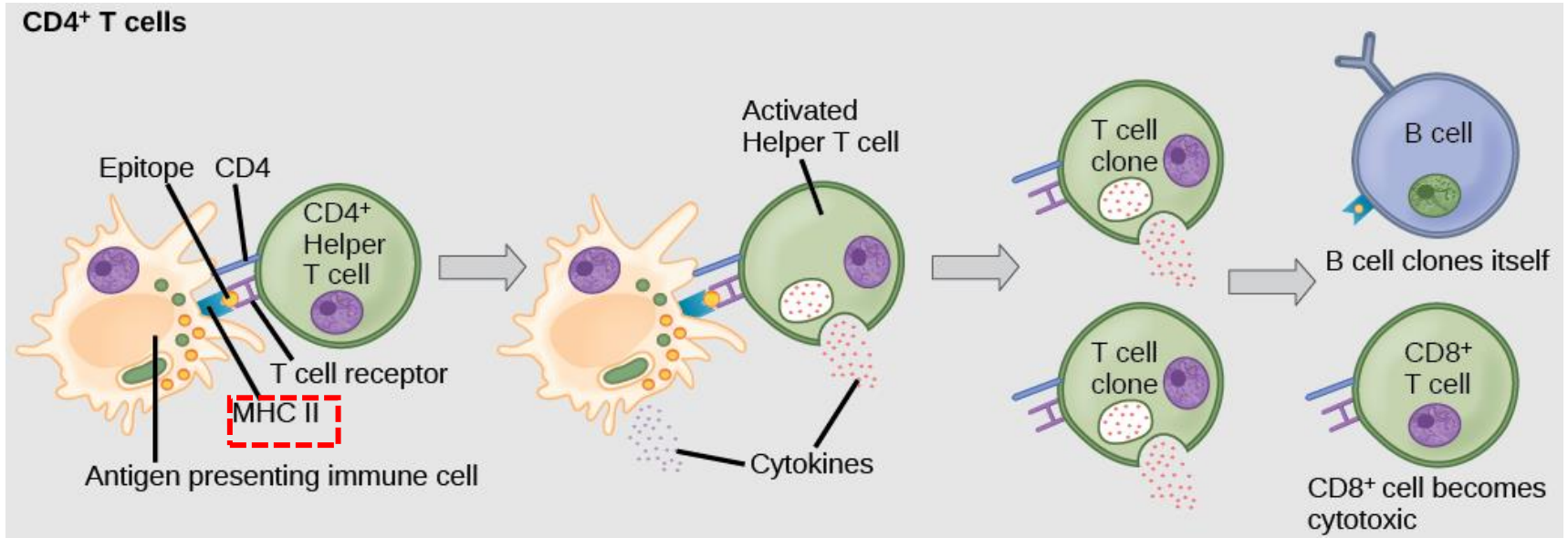
The Good of HLA

Activation of Adaptive Immune Response

Ag presentation by MHC Class I



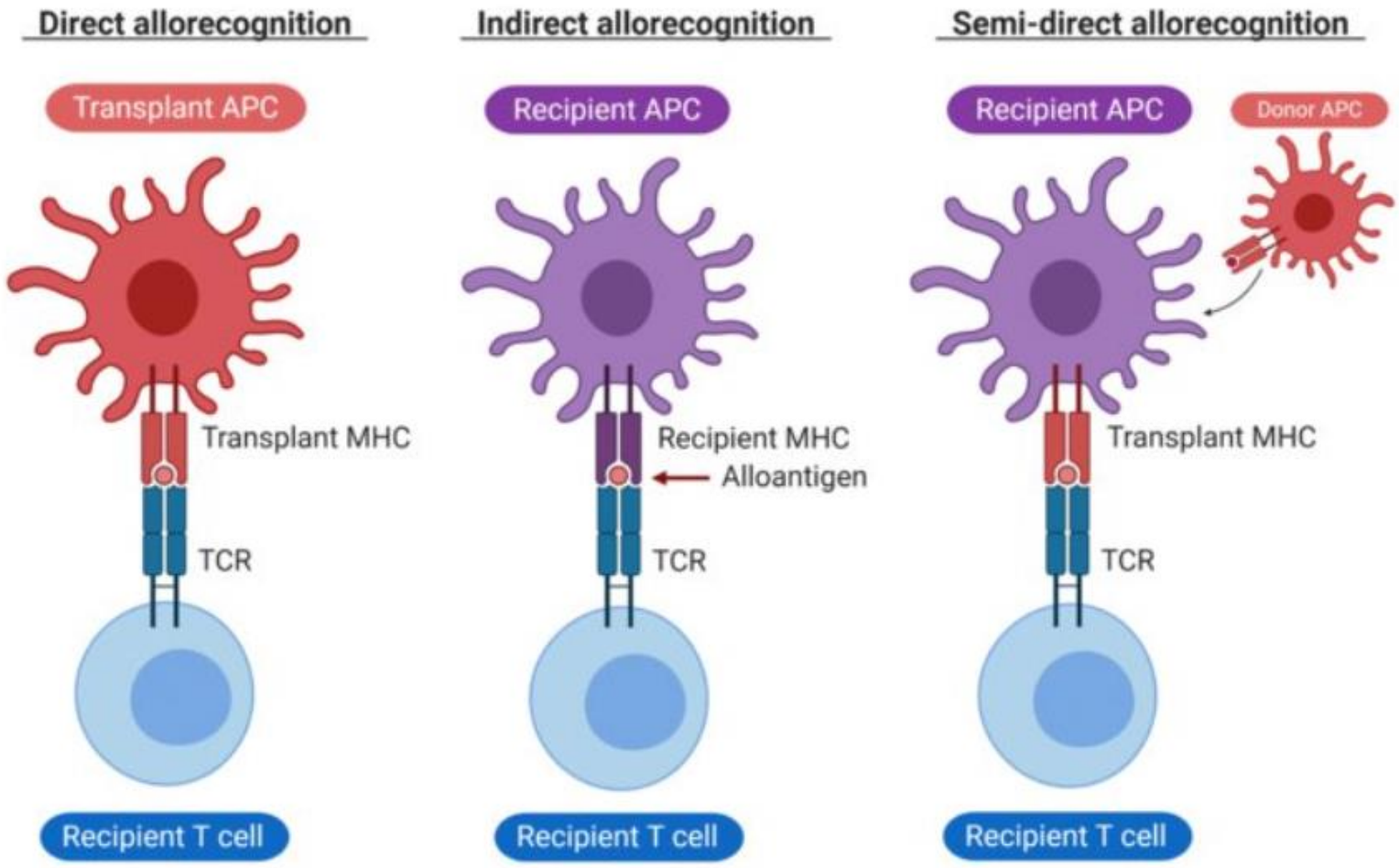
Ag presentation by MHC Class II



Molnar, C., & Gair, J. (2015). Concepts of Biology – 1st Canadian Edition.

The Bad of HLA

Mechanism of allo-response to HLA in transplant



Demkes EJ, et al. J Cardiovasc Transl Res. 2021;14(1):88-99.

Testing for HLA Antigens/Alleles

HLA Genotyping for Transplantation

HLA Matched Recipient/Donor Pair			
		Recipient	*Donor
HLA-A	Allele-1	02:01	02:01
	Allele-2	23:01	23:01
HLA-B	Allele-1	39:01	39:01
	Allele-2	44:03	44:03

HLA Mismatched Recipient/Donor Pair			
		Recipient	*Donor
HLA-A	Allele-1	02:01	03:01
	Allele-2	23:01	68:01
HLA-B	Allele-1	39:01	51:01
	Allele-2	44:03	15:02

*Donor HLA type used for example only

The Bad of HLA

HLA & Disease Association

HLA Antigen	Disease
HLA A3	Hematochromatosis
HLA A29	Birdshot chorioretinopathy
HLA B27	Ankylosing Spondylitis
HLA B51	Behcet's Disease
HLA DQ6	Narcolepsy
HLA DQ2 & DQ8	Celiac Disease

Fiorillo MT, et. al. Front Immunol. 2017 Nov 7;8:1475.

Liu B, et. al. Immun Inflamm Dis. 2021;9(2):340-350.

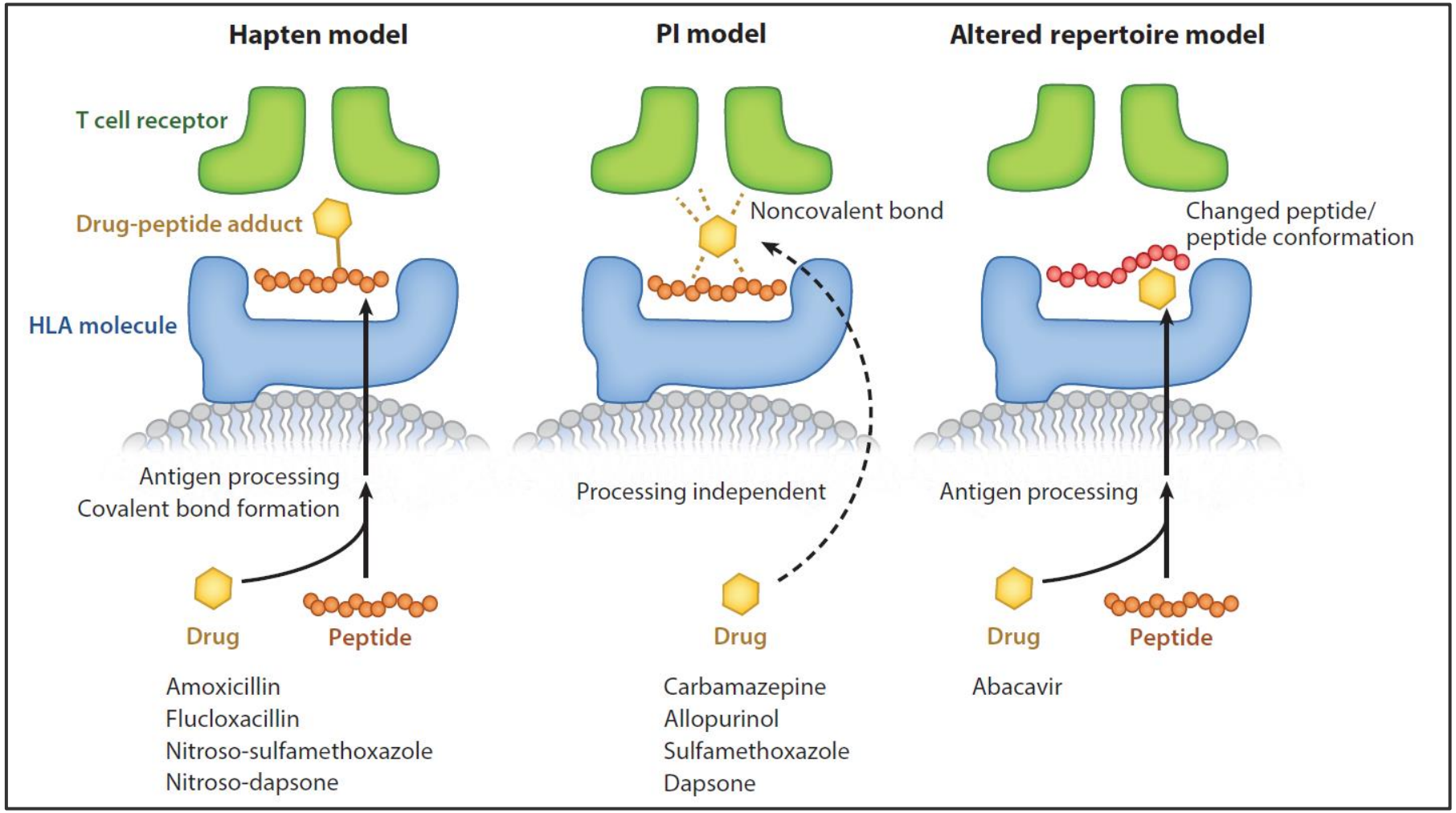
The Bad of HLA

HLA & Drug Hypersensitivity

HLA	Drug
B*58:01	Allopurinol
B*57:01	Abacavir
B*13:01	Dapsone
B*15:02	Carbamazapine
A*68:01	Lamotrizine
DRB1*13:02	Aspirin

Kloypan C, et. al. Pharmaceuticals (Basel). 2021;14(11):1077.

Proposed Mechanism of HLA Medicated Drug Hypersensitivity



Jaruthamsophon K, et al. Annu Rev Pharmacol Toxicol. 2022;62:509-529.

Companion Diagnostic

A companion diagnostic is:

a medical device, often an in vitro device, which provides information that is essential for the safe and effective use of a corresponding drug or biological product. The test helps a health care professional determine whether a particular therapeutic product's benefits to patients will outweigh any potential serious side effects or risks.

Companion diagnostics can:

- identify patients who are most likely to benefit from a particular therapeutic product;
- identify patients likely to be at increased risk for serious side effects as a result of treatment with a particular therapeutic product; or
- monitor response to treatment with a particular therapeutic product for the purpose of adjusting treatment to achieve improved safety or effectiveness.

https://www.fda.gov/medical-devices/in-vitro-diagnostics/companion-diagnostics

HLA as a Companion Diagnostic Tool

➢ Drug Hypersensitivity

- **HLA B*57:01- Abacavir**

  Recommended by FDA, EMA and CPIC

- **HLA B*58:01- Allopurinol**

  FDA- in genetically at-risk populations

  EMA and CPIC- Testing not recommended

- **HLA B*15:02- Carbamazepine**

  (Recommended by FDA, EMA and CPIC in specific population

  (Han Chinese, Thai and Asian population at genetic risk)

➢ Decision to use targeted drug

- **A*02:01- tebantafusp in metastatic uveal myeloma**

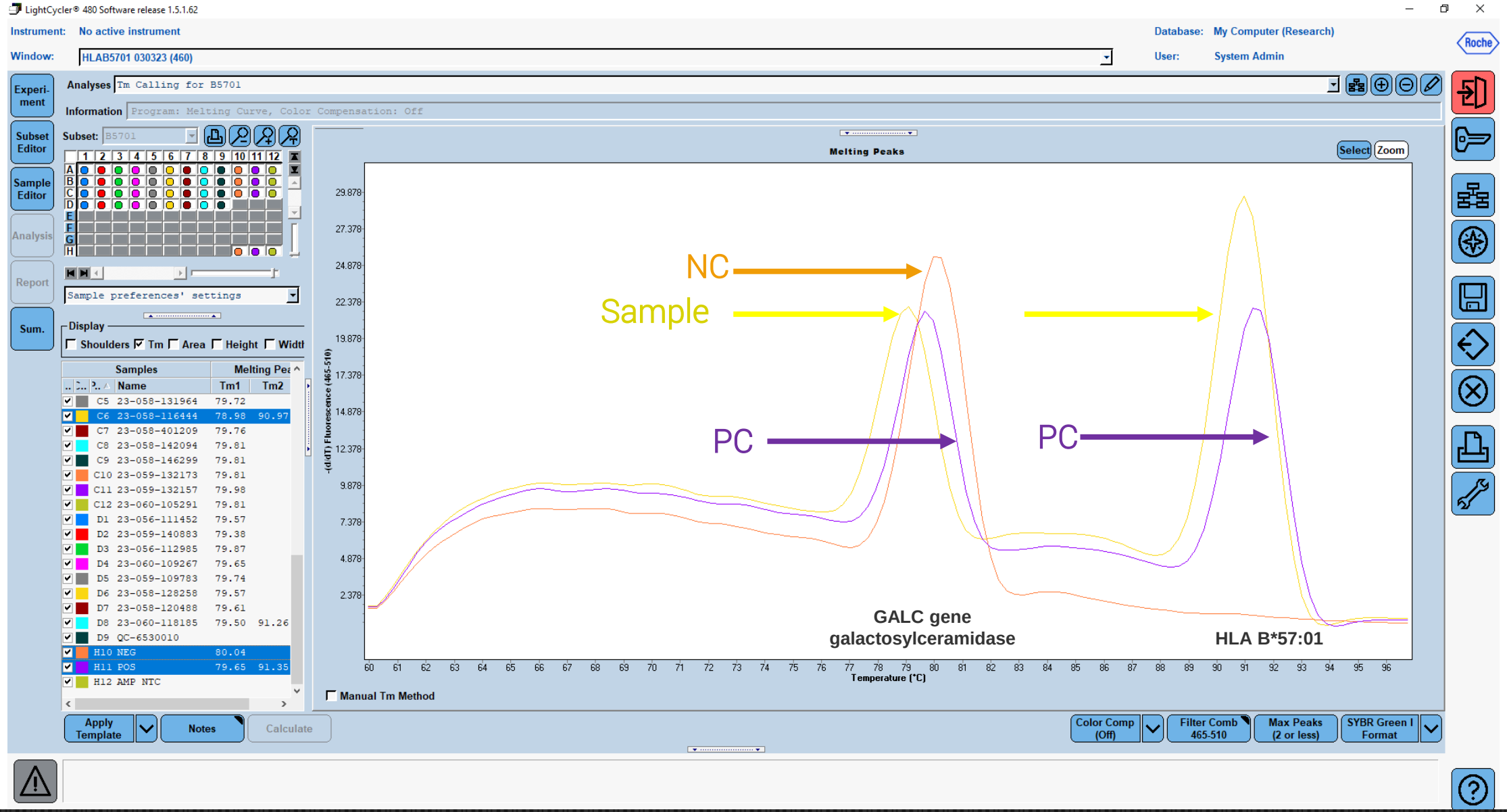
***FDA- U.S. Food and Drug Administration ; CPIC- Clinical Pharmacogenetics Implementation Consortium ; European Medicines Agency**

Methods of HLA Typing

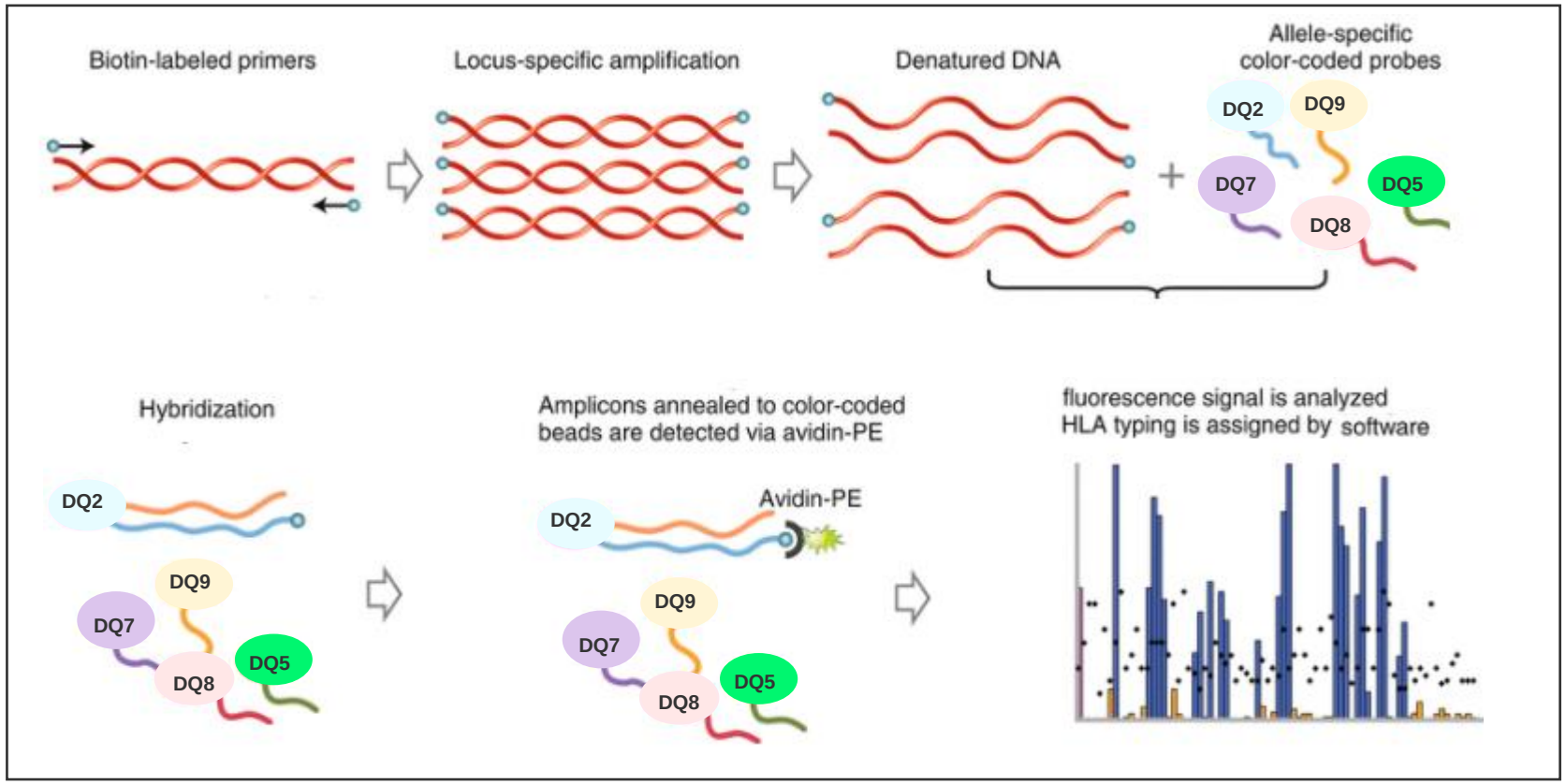
Melting Curve Analysis by Real time PCR

- **Melting temperature (Tm): at which 50% of the amplicon is single-stranded and 50% double-stranded.**
- **Intercalating fluorescent dye is used to non-specifically bind to double-stranded DNA only.**
- **Primers specific to targeted alleles and a reference gene, (GALC), are used.**
- **At the end of the PCR cycles, a melting curve is performed.**
- **Targeted approach; provides only qualitative results; gene dose can not be determined.**

Representative image for a sample positive for HLA B*57:01



HLA Testing by Sequence Specific Oligonucleotide (SSO)



Adapted and modified from -https://basicmedicalkey.com/

Limitation:

- **Due to the probe design for Exon 2 (PBD) domain only,**
- **Sometimes ambiguous results.**
- **Results reported out as allele code and string of alleles.**

HLA Testing by Massive Parallel Sequencing

Image Adapted from Omixon Biocomputing Ltd.

- **High throughput method.**
- **High resolution allele result without ambiguity.**
- **Higher cost of instrumentation, skilled staff.**

Challenges

Interpretation of test result

- **Low positive predictive value**
- **High negative predictive value**
- **Importance of the clinical context**
- **HLA typing methods with different resolution (B*57:01 Vs B57*02)**

Cost effectiveness

- **Cost effectiveness in population with low frequency of allele**

e.g. HLA B*15:02 testing for Carbamazepine

Global and population frequencies of rs144012689, *HLA-B*15:02*, and *HLA-B*15:13*.

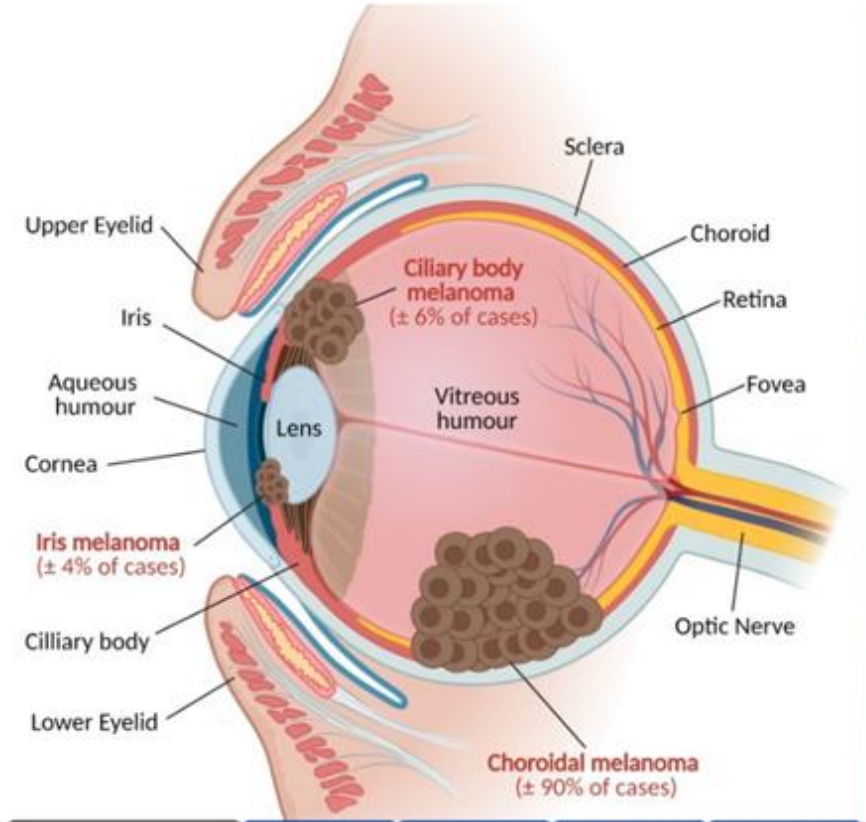
Allele	Global Populations	African	Asian	Caucasian	Hispanic
*HLA-B*15:02*	0.91% (*n* = 2,453,203)	0.21% (*n* = 41,314)	3.92% (*n* = 560,842)	0.001% (*n* = 1,325,156)	0.03% (*n* = 417,406)

Clinical Pharmacogenetics Implementation Consortium (CPIC); Fang H et al. Front Pharmacol. 2019

HLA as a Companion Diagnostic Tool

Novel Therapies in Cancer

Uveal Melanoma



UVEAL MELANOMA INITIATING MUTATIONS	GNAQ (≈ 50%)	GNA11 (≈ 45%)	CYSLTR2 (≈ 4%)	PLCB4 (≈ 2,5%)
"SECOND HIT" MUTATIONS WITH IMPACT IN THE PROGNOSIS	EIF1AX (≈ 13%)	SF3B1 (≈ 23%)	SRSF2 (≈ 4%)	BAP1 (≈ 33%)

Common symptoms of patients with uveal melanoma
- Blurred or distorted vision
- Loss of visual fields
- Photopsia
- Changes in the colour or appearance of a new lesion in the iris

*Nearly 1/3 of UM patients are asymptomatic: the disease is detected in routine ophthalmological check-up or screening for other eye conditions

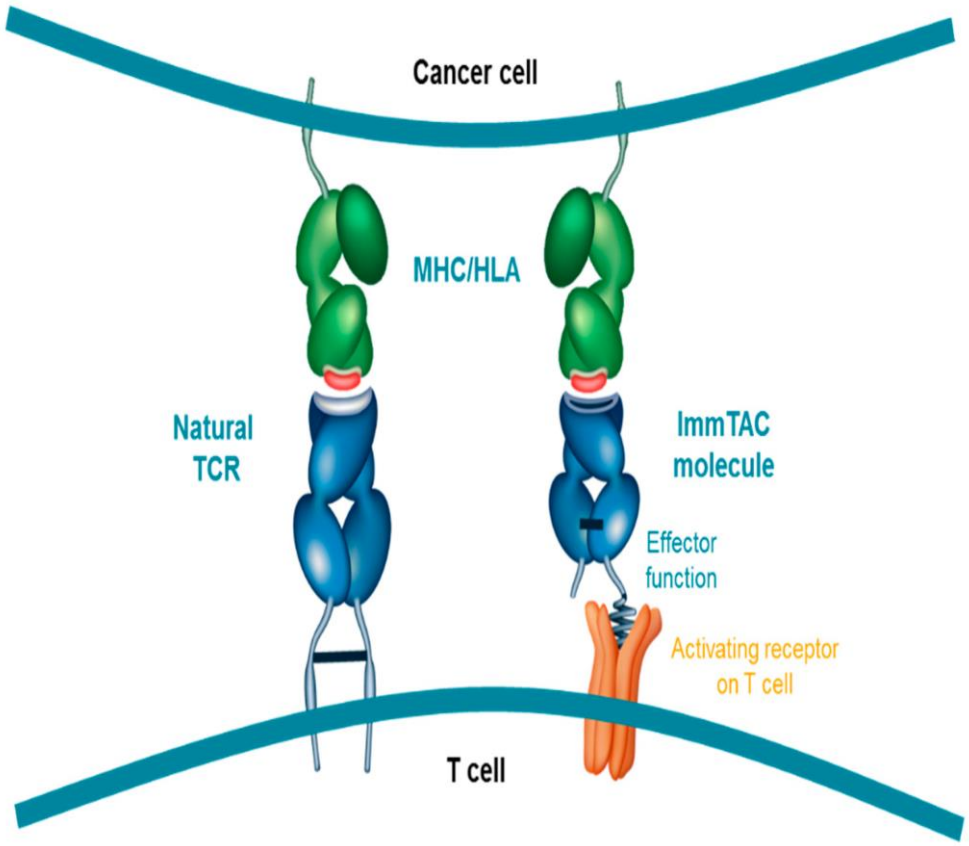
Presenting complications in patients with uveal melanoma
- Exudative retinal detachment
- Glaucoma
- Intraocular hemorrhage
- Cataracts
- Changes in the cornea, including edema and band keratopathy
- Vision loss
- Metastases, especially to the liver

Established risk factors for the development of uveal melanoma
- Age between 50 and 70 years
- Fair skin colour
- Light-coloured eyes (blue or grey)
- Sensitivity to sunburn
- Multiple skin naevi
- Northern European ancestry
- Congenital ocular melanocytosis
- Ocular melanocytoma
- Family history of cutaneous or uveal melanoma
- BAP1-tumour predisposition syndrome
- Germline mutations in PALB2, MLH1 or MBD4

Lamas NJ, et al. Cancers (Basel). 2021;14(1):96.

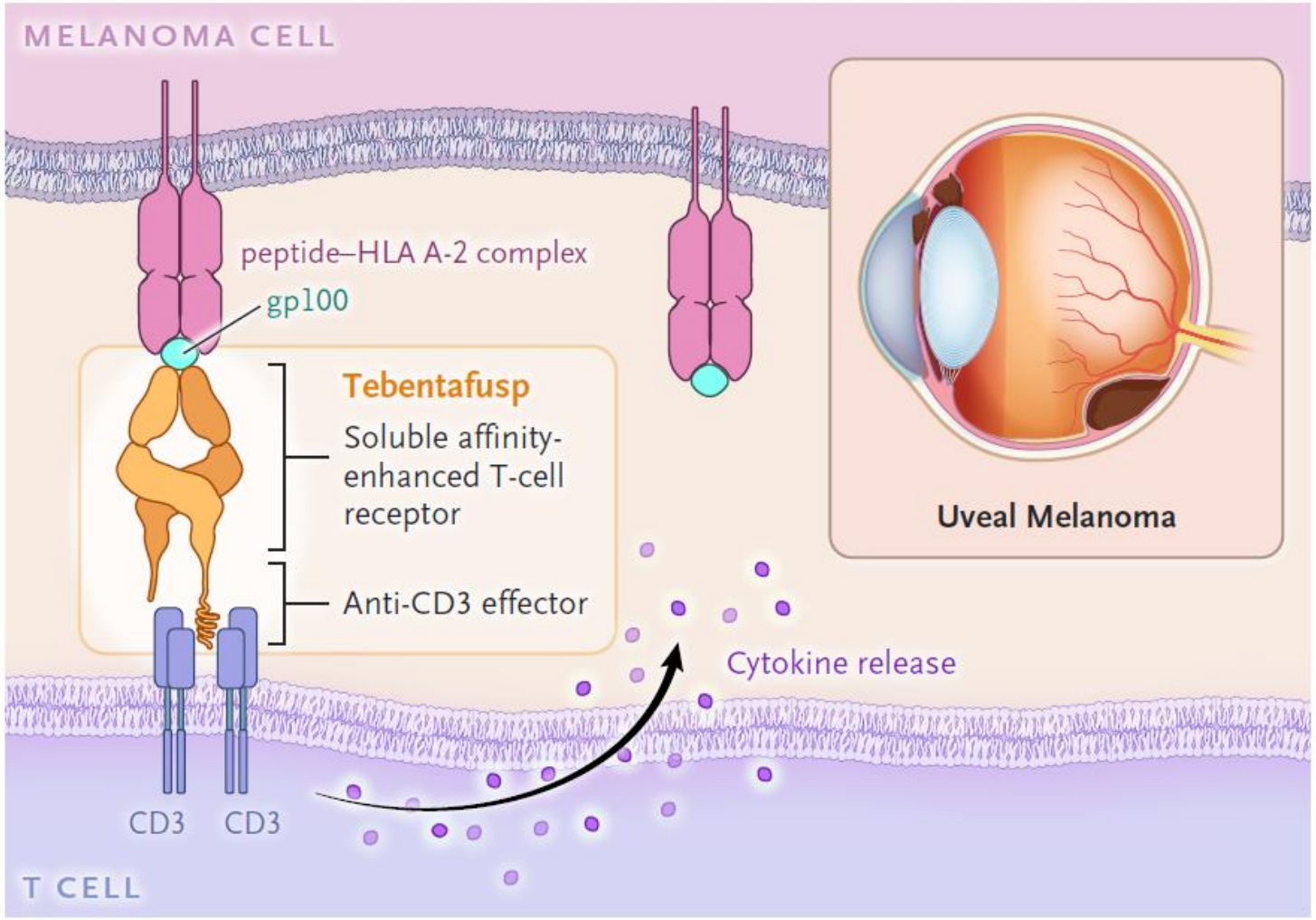
Tebentafusp - tebn

- ~50% of patients with uveal melanoma develop metastatic disease.
- In patient with metastatic disease overall survival is 1 year.
- Tebentafusp – tebn, is a T cell receptor (TCR) immunotherapy.
- Bispecific gp100 peptide-HLA-directed CD3 T cell engager.
- US- FDA approved Tebentafusp - tebn on January, 2022.
- Unresectable or metastatic uveal melanoma
- HLA-A*02:01-positive adult patients.



Damato BE, et al. Cancers 2019, 11(7), 971

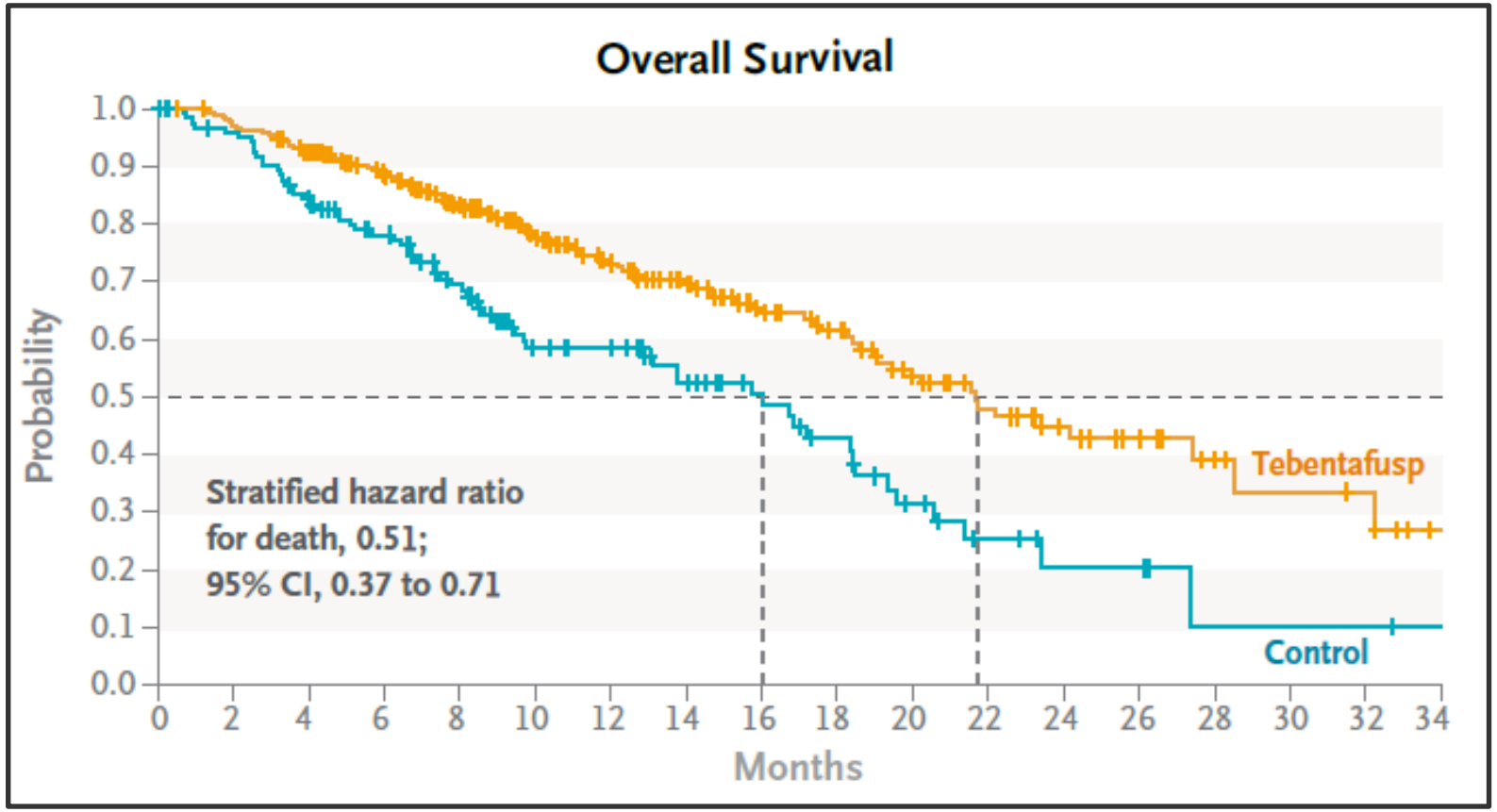
Mechanism of action of tebentafusp-tebn



Nathan P et al. DOI: 10.1056/NEJMoa2103485

Survival Benefit With Tebentafusp - tebn

- 378 patients with metastatic Uveal melanoma.
- N=252 previously untreated HLA-A*02:01–positive patients.
- Controls received either pembrolizumab, ipilimumab, or dacarbazine
- The primary end point was overall survival.



Nathan P et al. N Engl J Med. 2021;385(13):1196-1206.

Mature Protein Sequences of HLA A2 Alleles

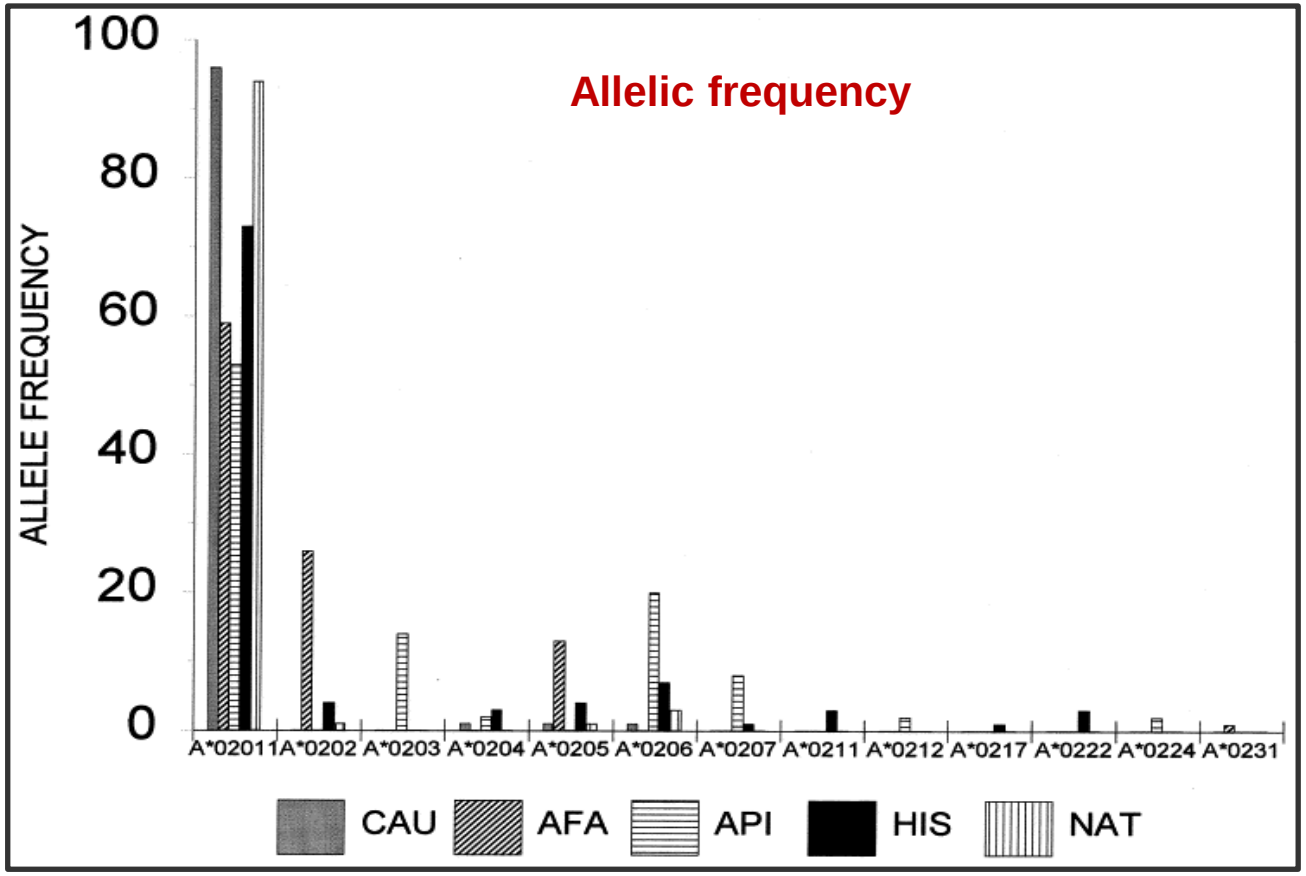
```
AA Pos.                10        20        30        40        50        60        70        80        90       100
A*02:01:01:01  GSHSMRYFFT SVSRPGRGEP RFIAVGYVDD TQFVRFDSDA ASQRMEPRAP WIEQEGPEYW DGETRKVKAH SQTHRVDLGT LRGYYNQSEA GSHTVQRMYG
A*02:02:01:01  ---------- ---------- ---------- ---------- --R------- ---------- ---------- ---------- ---------- ----L-----
A*02:03:01:01  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
A*02:04:01     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------M---
A*02:05:01:01  --------Y- ---------- ---------- ---------- --R------- ---------- ---------- ---------- ---------- ----L-----

AA Pos.                110       120       130       140       150       160       170       180       190       200
A*02:01:01:01  CDVGSDWRFL RGYHQYAYDG KDYIALKEDL RSWTAADMAA QTTKHKWEAA HVAEQLRAYL EGTCVEWLRR YLENGKETLQ RTDAPKTHMT HHAVSDHEAT
A*02:02:01:01  ---------- ---------- ---------- ---------- ---------- -----W---- ---------- ---------- ---------- ----------
A*02:03:01:01  ---------- ---------- ---------- ---------- ---------T- -E---W---- ---------- ---------- ---------- ----------
A*02:04:01     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
A*02:05:01:01  ---------- ---------- ---------- ---------- ---------- -----W---- ---------- ---------- ---------- ----------

AA Pos.                210       220       230       240       250       260       270       280       290       300
A*02:01:01:01  LRCWALSFYP AEITLTWQRD GEDQTQDTEL VETRPAGDGT FQKWAAVVVP SGQEQRYTCH VQHEGLPKPL TLRWEPSSQP TIPIVGIIAG LVLFGAVITG
A*02:02:01:01  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
A*02:03:01:01  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
A*02:04:01     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
A*02:05:01:01  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

AA Pos.                310       320       330       340
A*02:01:01:01  AVVAAVMWRR KSSDRKGGSY SQAASSDSAQ GSDVSLTACK V
A*02:02:01:01  ---------- ---------- ---------- ---------- -
A*02:03:01:01  ---------- ---------- ---------- ---------- -
A*02:04:01     ---------- ---------- ---------- ---------- -
A*02:05:01:01  ---------- ---------- ---------- ---------- -
```

https://www.ebi.ac.uk/cgi-bin/ipd/pl/hla/align.cgi

Why HLA - A2 ?

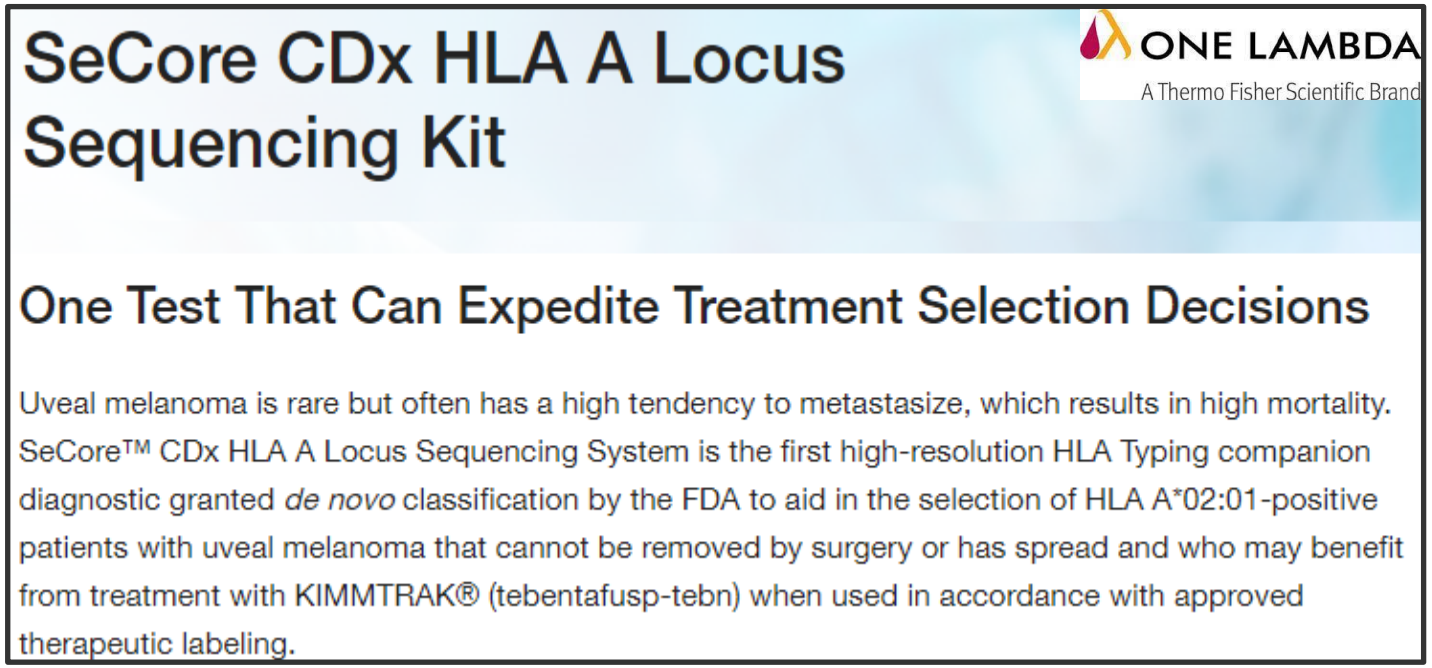
Phenotypic frequency of HLA-A2 within the study population

Population	Population size	#HLA-A2 positive	
Caucasian	61,655	30,596	(49.6%)
African-American	8,288	2,864	(34.6%)
Asian/Pacific Islander	2,275	819	(36.0%)
Hispanic	4,879	2,286	(46.9%)
Native American	5,882	2,922	(49.7%)
Total	82,979	39,487	(47.6%)



Ellis JM, et al. Hum Immunol. 2000;61(3):334-340.

The First HLA typing companion diagnostic test granted *de novo* classification by FDA



The device is intended to be used as a companion diagnostic (CDx) to aid in the selection of HLA A*02:01 patients with unresectable or metastatic uveal melanoma who may benefit from treatment with tebantafusp-tebn.

Summary and Future Perspectives

- HLA plays a significant role in Immune regulation.

  -balance of protective Immunity and tolerance.

- Some HLA alleles may pose a risk of autoimmune disease or ADR.
- HLA may be utilized in development of the safer Immunotherapies.
- Therapies that can be universally applied to all populations.

  -Via targeting common HLA antigens HLA A2; HLA B7 etc.

ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.