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IDENTIFICATION OF GENETIC FACTORS PREDISPOSING TO CHEMOTHERAPY AND IMMUNOTHERAPY TOXICITY

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Talk outline

- Background of research project
- Identification of genetic factors predisposing to fluoropyrimidine toxicity including cardiac toxicity
- Identification of genetic factors predisposing to ICI toxicity
- Summary and future directions

Introduction

- Adverse events from cancer therapy can cause chronic morbidity, impact patient's QOL and increase healthcare burden
- Predictive biomarkers of toxicity can help identify patients at risk
- Germline genetic variation as determinants of toxicity risk, eg *DPYD* (5-FU), *UGT1A1* (irinotecan)
- Hypothesis: Inherited genetic variation can influenced development of toxicity caused by chemotherapy and immunotherapy

Introduction- methods to identify risk variants

Tagging SNP arrays

- Analyse specific genomic regions
- GWAS to identify associations between genetic loci and phenotypic traits
- Cost-effective c/o sequencing

Whole exome sequencing

- Limited to coding regions
- More cost-effective

Whole genome sequencing

- Comprehensive genotyping
- Assess rare variants
- Costly & complex to analyse

Illumina global screening array

https://www.illumina.com/products/by-type/microarray-kits/infinium-global-screening.html

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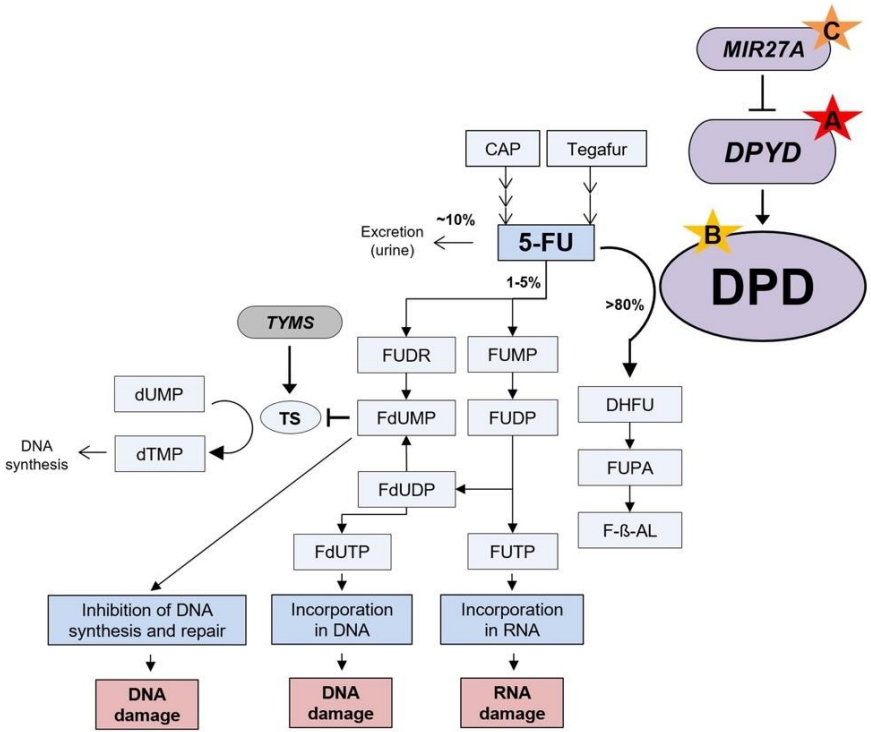
CTCAE toxicity grading system

CTCAE Grade	Description
1	Asymptomatic/mild symptoms. Clinical or diagnostic observations only. Intervention not needed
2	Moderate/minimal symptoms. Local or non-invasive intervention required. Limiting age-appropriate instrumental ADL
3	Severe/medically significant but not immediately life-threatening. Hospitalization or prolonged hospitalization needed. Disabling and limiting self-care ADL
4	Life-threatening and urgent intervention indicated.
5	Death

Part 1: Genetic Determinants of FP toxicity



- mir27A involved in post-transcriptional regulation of DPD. Overexpression of mir27A reduces DPD expression
- *MIR27A* variant rs895819 modifies early toxicity risk in *DPYD* risk variant carriers[1,2]

CPIC *DPYD* variants

c.1905+1G>A/*2A
c.2846A>T
c.1679T>G
c.1129-5923C>G/HapB3

However current screening tests has limited sensitivity in identifying all patients at risk

[1]Meulendijks et al. *Int. J. Cancer*, 2016
[2]Amstutz et al, *Clin Cancer Res*, 2015

Samples and workflow used in the analysis

930 patients on capecitabine from 2 Arms in QUASAR2 with genotyping and toxicity data

Genotyped using:
- Illumina tagging SNP arrays
- Imputation (1KG, UK10K reference panel)
- Illumina Exome Array, custom KASP targeted assay

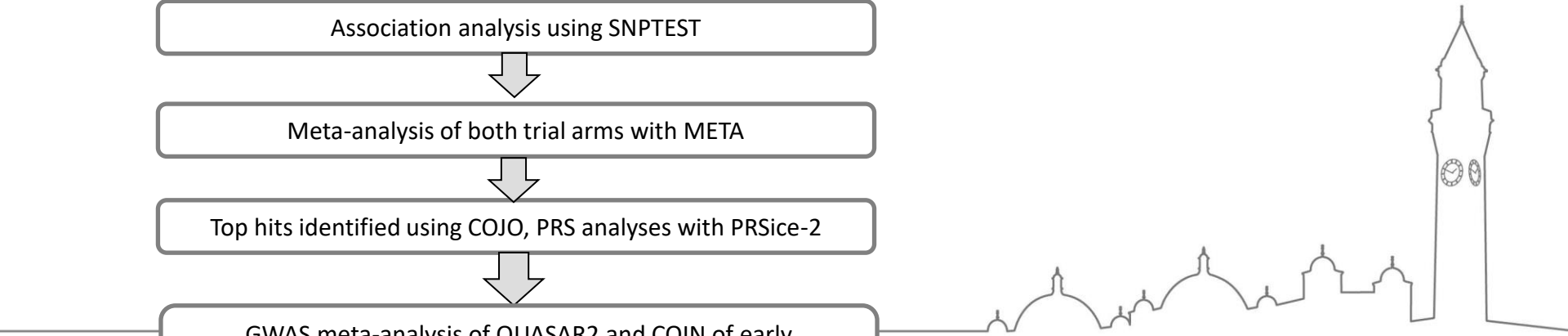
Binary coded variables generated for first 2 cycles/all cycles to identify CTCAE ≥gr 3 toxicity : diarrhoea, HFS, haematological (neutropenia + thrombocytopenia), mucositis+/-stomatitis, vomiting, global

Association analysis using SNPTEST

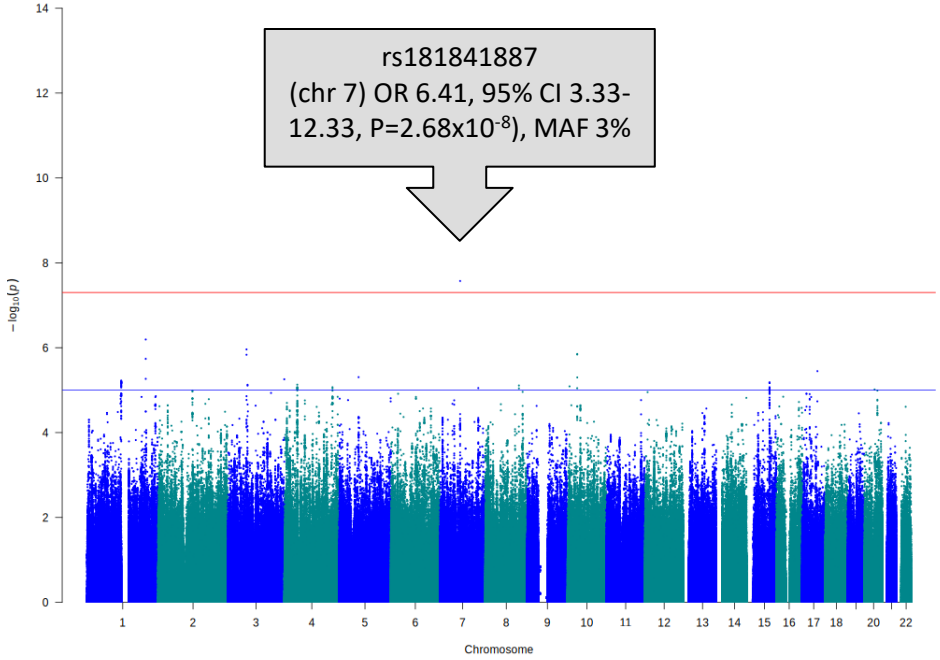
Meta-analysis of both trial arms with META

Top hits identified using COJO, PRS analyses with PRSice-2

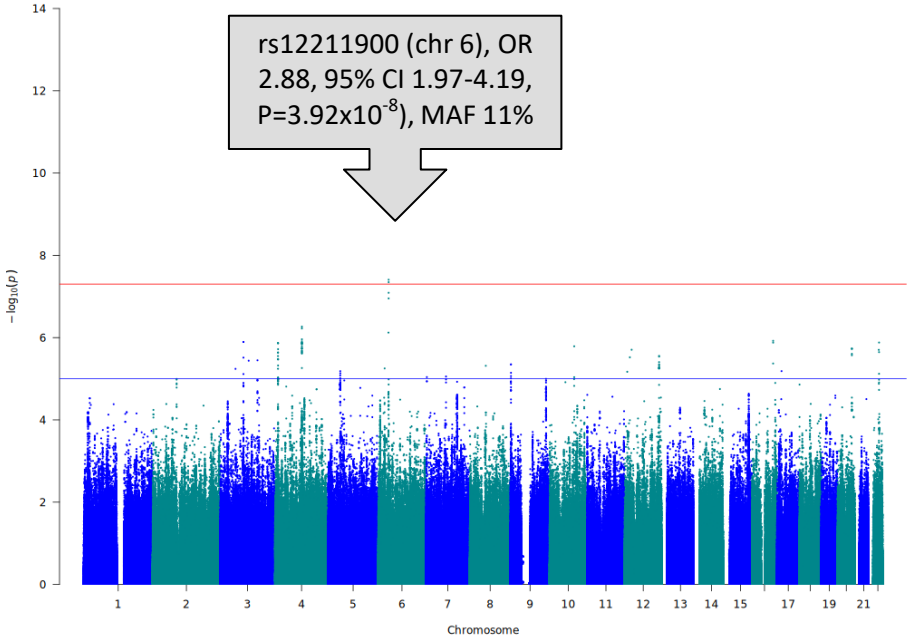
GWAS meta-analysis of QUASAR2 and COIN of early capecitabine toxicity



SNPs reaching GWAS significance in analysis of ≥grade 3 Capecitabine toxicity in QUASAR2



GWAS of early ≥grade 3 HFS



GWAS of all cycle ≥grade 3 diarrhoea

Meta-analysis of QUASAR2 & COIN

- Total 1928 CRC patients treated with capecitabine
- 4 SNPs associated with early ≥grade 3 toxicity with GWAS significance ($P \leq 5x10^{-8}$)

Toxicity	Chr	rsIDs	Freq	OR	95% CI	P value	Mapped/nearest genes
Mucositis	8	rs117241924	0.02	28.82	8.81-94.3	2.74E-08	KHDRBS3, LINC02055
Haem	21	rs140805091	0.02	14.21	5.48-36.86	4.85E-08	APP
Vomiting	5	rs146950191	0.02	21.34	7.21-63.16	3.22E-08	AC034220.3
Global	1	rs2477888	0.08	2.16	1.64-2.83	3.71E-08	RP11-418J17.3

Association between *MIR27A* rs895819 and capecitabine tox

Mir27A meta-analysis of QUASAR2 and a Dutch cohort

Early Toxicity	Capecitabine Only (N=2357)			
	No. of grade 3-4 cases	OR	95% CI	P value
Diarrhoea	**158**	**1.35**	**1.06-1.72**	**0.016**
Haem	91	0.94	0.68-1.31	0.720
HFS	121	1.2	0.90-1.58	0.213
Mucositis	28	1.23	0.70-2.15	0.472
GI	**175**	**1.33**	**1.05-1.68**	**0.017**
Global	**345**	**1.23**	**1.03-1.46**	**0.021**

rs895819 significantly associated with early ≥grade 3 diarrhoea, GI and global events

Stratified analysis according to *DPYD* status for rs895819

Toxicity	DPYD risk allele carriers (n=130)				DPYD wild type (n=2306)			
	OR	Lower CI	Upper CI	P-value	OR	Lower CI	Upper CI	P- value
Diarrhoea	1.24	0.43	3.57	0.68	1.33	1.04	1.71	0.02
GI	0.88	0.35	2.20	0.79	1.30	1.03	1.65	0.03
Global	0.88	0.40	1.92	0.75	1.23	1.03	1.47	0.02

Contrary to previous studies, rs895819 was significantly associated with an increased risk of early GI and global toxicity in *DPYD* wild type patients but not in the smaller subset of *DPYD* variant carriers.

No evidence that carrying the rs895819 alternate allele modifies risk in *DPYD* carriers in this analysis.

Identifying novel genetic determinants of FP cardiac tox

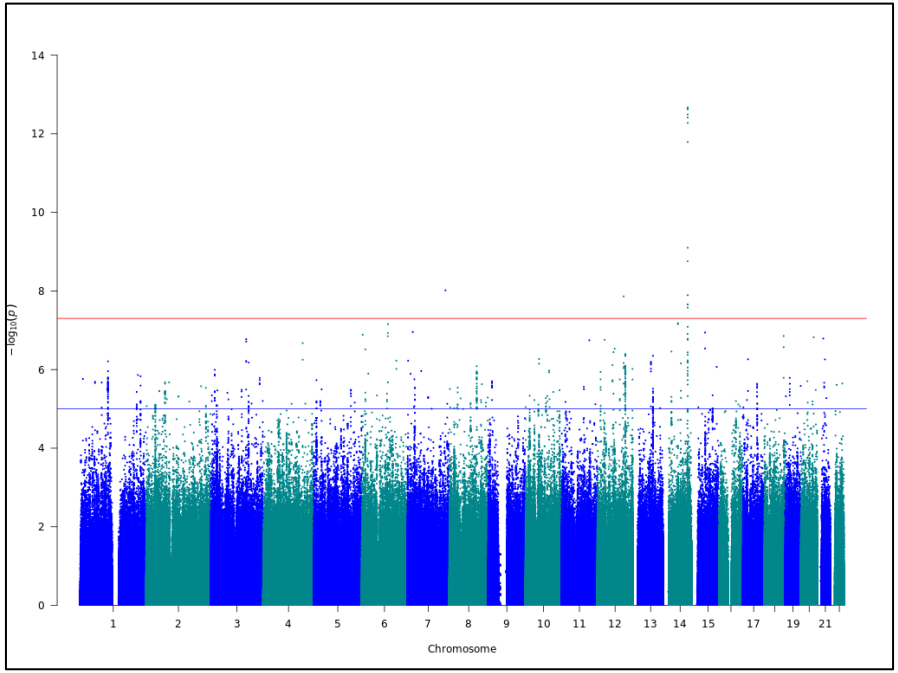
- 5FU cardiotoxicity can occur in up to ~35% of patients, ~2% can be serious/fatal
- Exact pathophysiology unknown
- Variants mapping to *DGLUCY* have been linked with cardiac toxicity in QUASAR2 (Unpublished data)
- Testing *DPYD* variants association with FP cardiotox in Q2:

DPYD variant	MAF	OR	95% CI	P value
c.1129–5923C>G/HapB3*	**0.02**	**4.28**	**1.40-13.04**	**0.03**

Meta-analysis of 3 studies of 5-FU cardiotox (N=5604)

Study	No. of patients with cardiac toxicity
QUASAR2	29
COIN	46
SCOT	39

GWAS meta-analysis of cardiac toxicity (additive model)

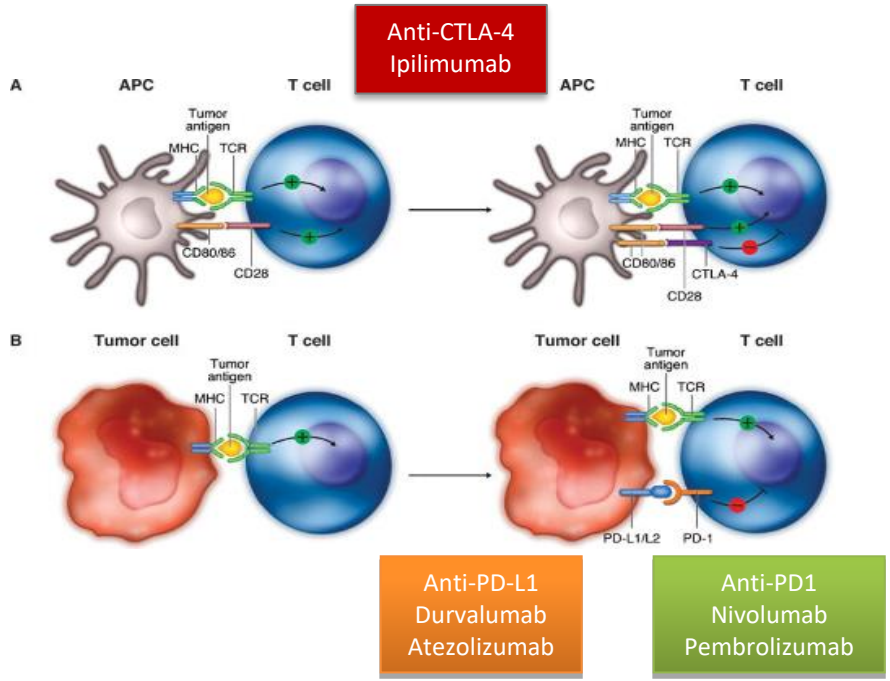


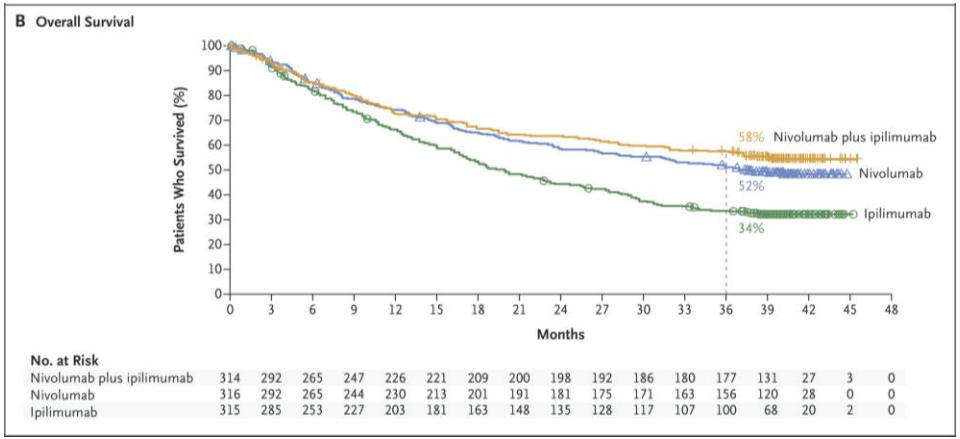
rsID	Chr	Pos	refA	Freq	OR	95% CI	P value	Consequences	Mapped Genes
rs141495755 ***	7	144498917	T	0.02	5.63	3.12-10.15	9.64E-09	Intronic	TPK1
rs117095544 **	12	99233430	A	0.02	6.26	3.32-11.78	1.37E-08	intronic	ANKS1B

Summary for part 1

- GWAS from Q2 found 2 SNPs associated with ≥grade 3 early HFS and any cycle diarrhoea at GWAS significance but need validation in an independent cohort. *DPYD* variants have low predictability of diarrhoea so this SNP could be clinically valuable.
- Meta-analysis of Q2 and COIN identified 4 GWAS sig variants associated with early serious capecitabine tox.
- *MIR27A* common variant rs895819 significantly increased the risk of early ≥gr3 diarrhoea and global toxicity but not in *DPYD* low function/no function allele carriers.
- *DPYD* HapB3 and novel variants identified from meta-analysis could also contribute to future screening panels of 5-FU cardiotoxicity.

Part 2:
Identification of genetic factors predisposing to ICI toxicity





Wolchok et al, *NEJM*, 2017

At 36 months f/up, over half of patients are still alive from nivo/ipi or nivo alone, but gr 3-4 treatment-related AE occurred in 59% (nivo/ipi), 21% (nivo), 28% (ipi)

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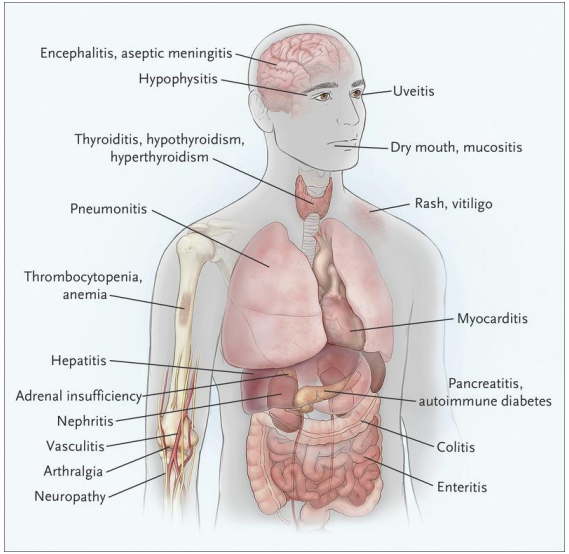
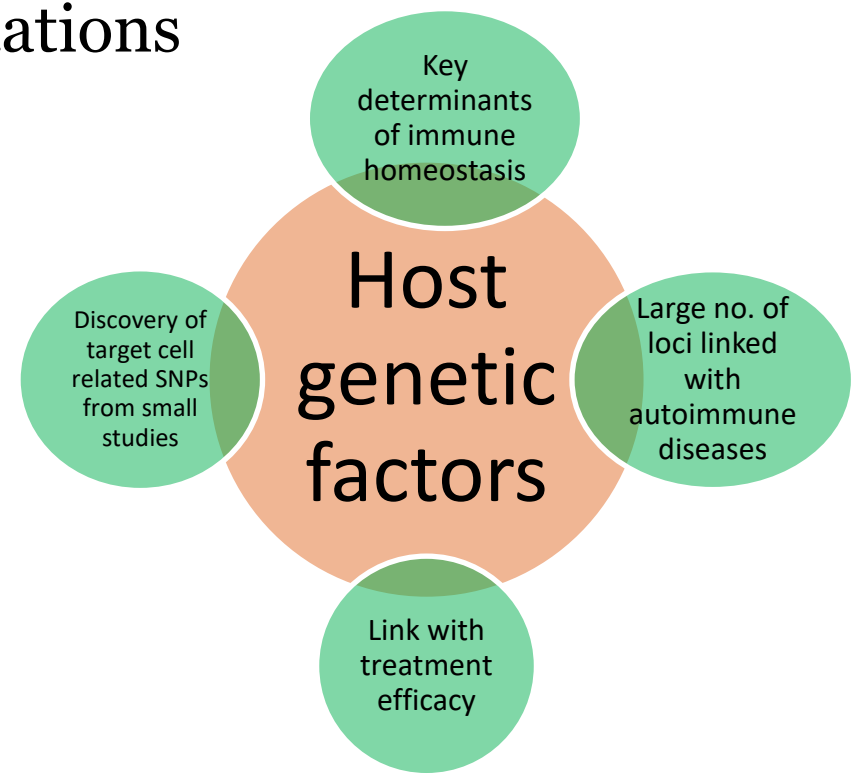
Broad spectrum of irAE manifestations





Currently unable to predict which patient is at risk of serious toxicity, but germline genetic variation could determine toxicity risk

Methods- ICI toxicity

Patient samples

Participants from ICI GENETICS (Prospective, retrospective + grade 3+ tox, relative with AID)

PIS & ICF

- Blood samples (EDTA)/saliva
- Patient & clinician questionnaire

Toxicity CRF at 3,6,12,24 months

Samples Processing

Samples/data logged on to REDCap

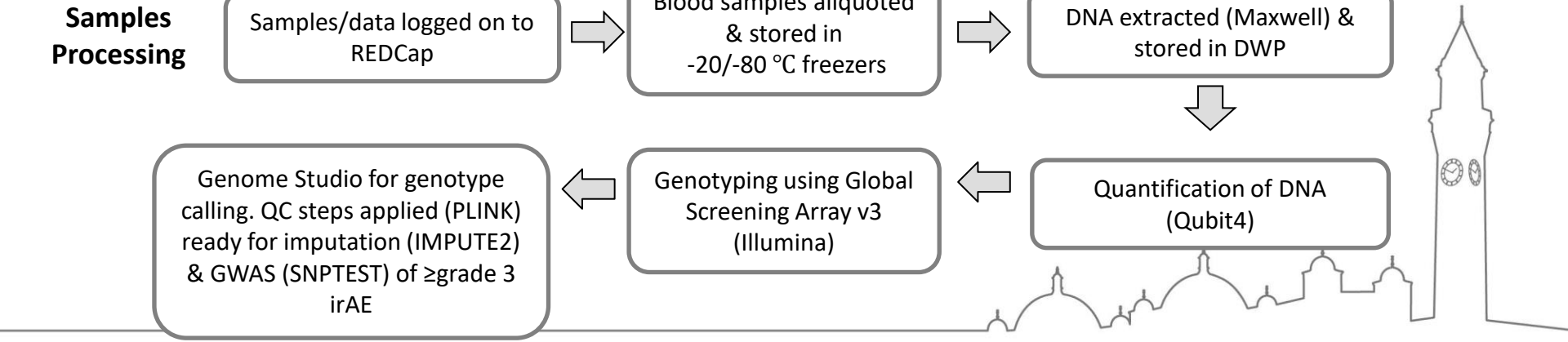
Blood samples aliquoted & stored in -20/-80 °C freezers

DNA extracted (Maxwell) & stored in DWP

Quantification of DNA (Qubit4)

Genotyping using Global Screening Array v3 (Illumina)

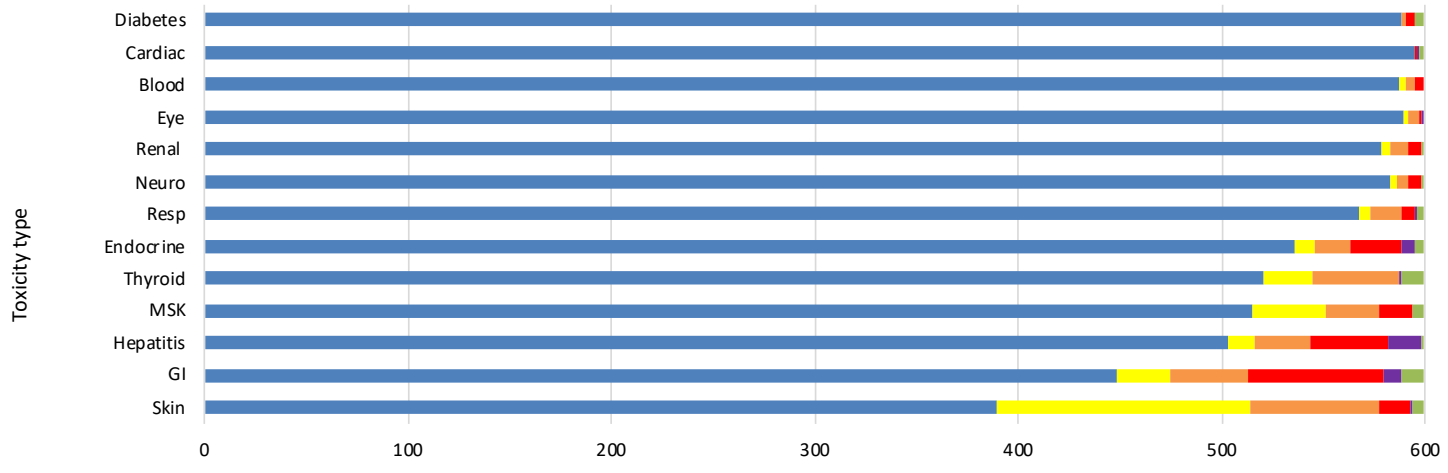
Genome Studio for genotype calling. QC steps applied (PLINK) ready for imputation (IMPUTE2) & GWAS (SNPTEST) of ≥grade 3 irAE



Baseline characteristics of discovery cohort

Demographics **Total N=599**	**No. of patients with grade 0-2 irAE (n=413, 69%)**	**No. of patients with grade 3-4 irAE (n=186, 31%)**
Sex		
Female	189, 46%	76, 41%
Male	224, 54%	110, 59%
Mean age (range)	66 (23-96)	61 (21-88)
Cancer type		
Skin/Melanoma	273, 66%	157, 84%
Lung/thoracic	96, 23%	17, 9%
Kidney	34, 8%	12, 6%
Head and neck	4, 1%	0
Bladder	2, 0.5%	0
Other	3, 0.7%	0
Unknown	1, 0.002%	0
ICI agent		
Anti-CTLA-4 + anti-PD1	95, 23%	130, 70%
Anti-PD1	268, 65%	42, 23%
Anti-PDL1	19, 5%	1, 0.5%
Anti-CTLA-4	11, 3%	2, 1.1%
Other (please specify)	1, 0.2%	0
Unknown	19, 5%	11, 6%
Concurrent Therapy		
Yes	17, 4%	5, 3%
No	395, 96%	181, 97%
Unknown	1	0

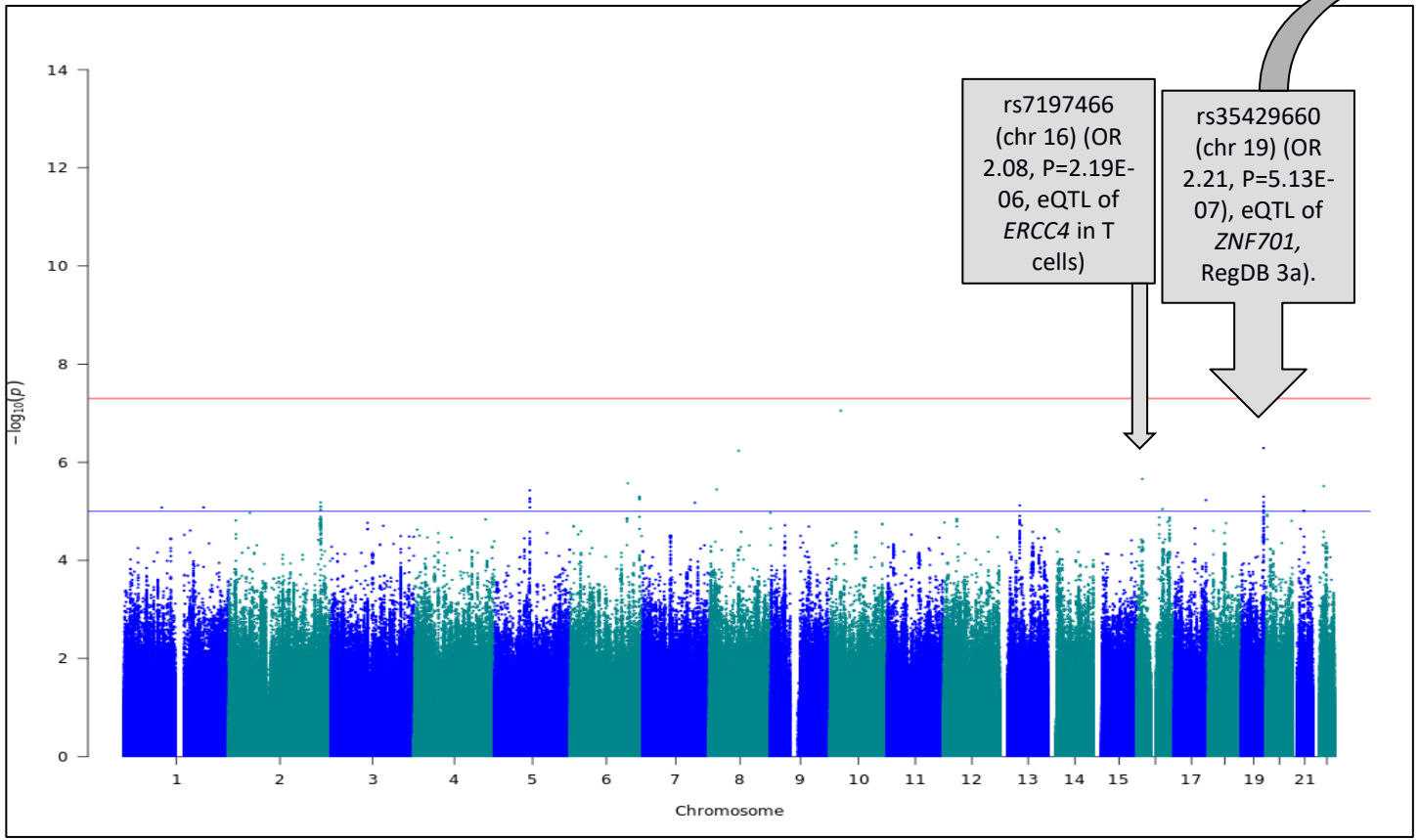
irAEs observed in the discovery cohort



	Skin	GI	Hepatitis	MSK	Thyroid	Endocrine	Resp	Neuro	Renal	Eye	Blood	Cardiac	Diabetes
Grade 0/No toxicity	389	448	503	515	520	536	567	583	578	589	587	595	588
Grade 1	125	27	13	36	25	10	6	3	5	3	3	0	0
Grade 2	63	38	27	26	42	17	15	5	8	5	5	0	2
Grade 3	16	66	39	17	0	25	7	7	7	1	4	1	5
Grade 4	1	9	16	0	1	7	1	0	0	1	0	1	0
NA	5	11	1	5	11	4	3	1	1	0	0	2	4

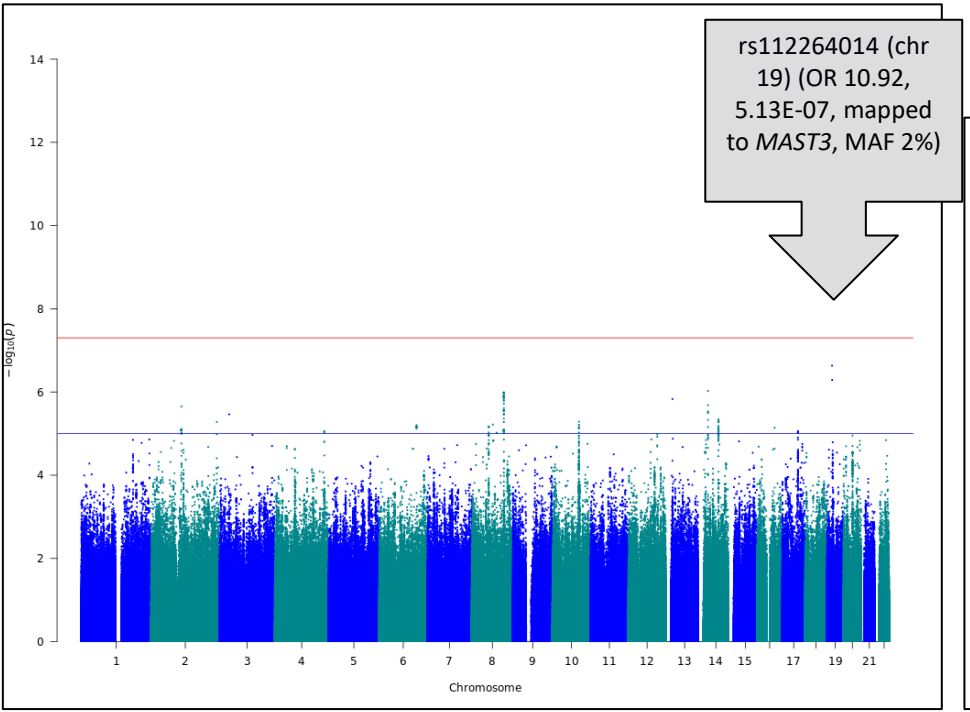
- Skin tox were the commonest any grade tox and GI tox formed the highest proportion of serious ≥gr 3 tox
- Powered at 80% to identify common variants (MAF 30%) with OR ≥1.9 & rare variants (MAF 10%) at OR ≥2.2

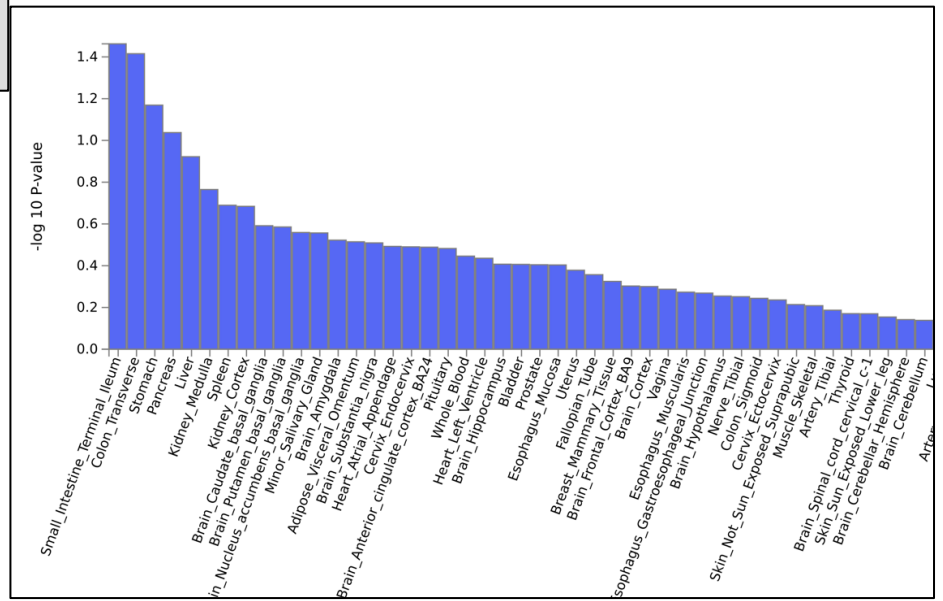
ICI induced any serious toxicity GWAS results



8 SNPs were associated with any serious irAE at $P \leq 5x10^{-6}$. Rs35429660 is an significant eQTL of *ZNF701* has the lowest RegulomeDB score 3a

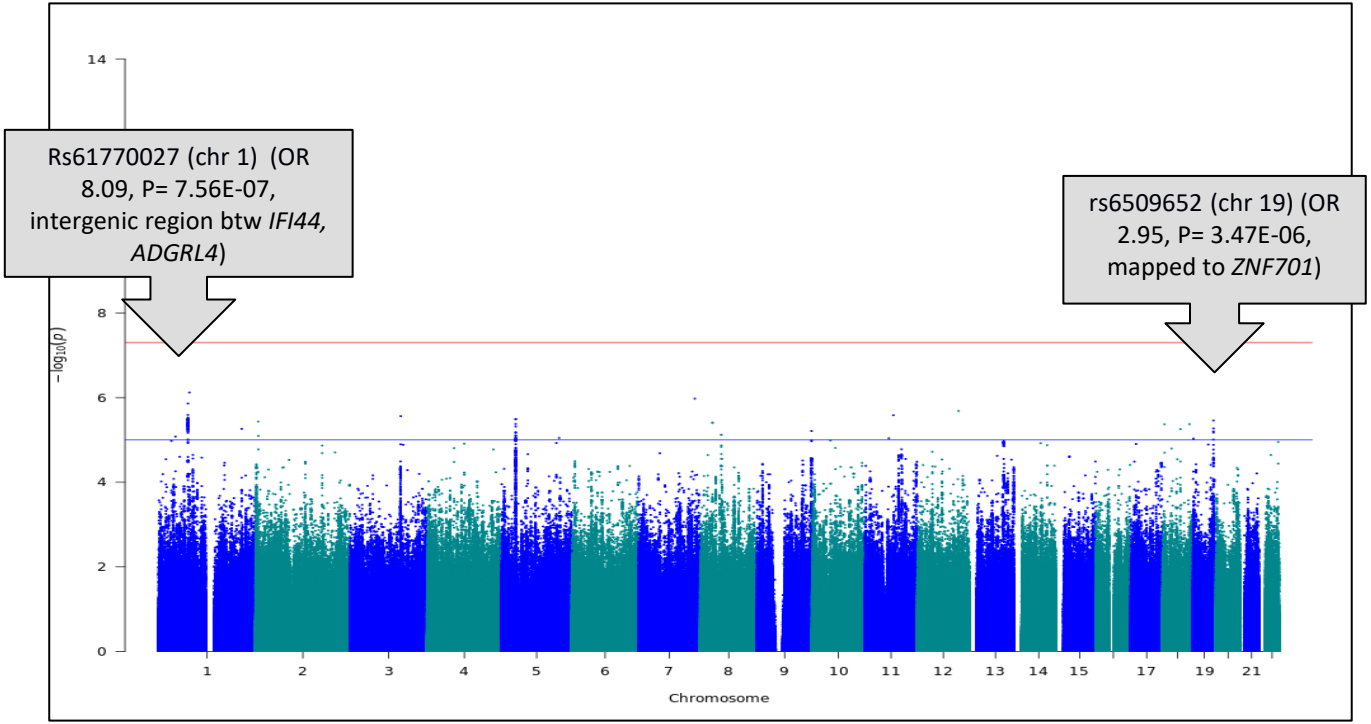
ICI induced ≥gr 3 Gastro-Intestinal toxicities GWAS results





GI toxicity associated genes were highly expressed in the tissues of the GI tract, including the terminal ileum of the small intestine (P=0.03), transverse colon (P=0.04) and stomach (P=0.07).

ICI induced ≥gr 3 hepatitis GWAS results



Top immune-related hepatitis SNP rs6509652 reduces expression of *ZNF701* in CD4 T cells

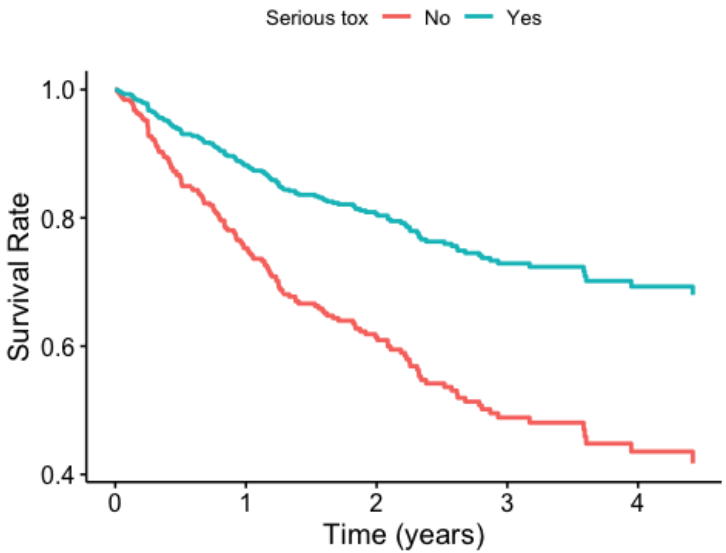
Validating our top hits

- Groha et al- GWAS of ICI toxicity in 1751 patients[3]
  - Only 23/80 of the top SNPs were available from their data, with 2 SNPs validating at P<0.05 in their cohort
  - **rs16906115** in an intron of IL-7 is their top hit associated with all grade irAE and is validated at P=0.049 in our GWAS of serious irAE
- Validation cohort of 103 ICI GENETICS serious tox cases + 2497 controls from UKBiobank
  - None of the top any serious irAE SNPs validated at P<0.05
  - One hepatitis SNP validated at P<0.05 with the same direction of effect

rsID	Toxicity risk allele	Discovery OR (95% CI)	Discovery P-value	Validation OR (95% CI)	Validation P-value	Meta P-value
rs77941834	T	5.17 (2.29-11.68)	2.74 E-06	2.20 (0.94-5.15)	0.047	3.95 E-05

[3] Groha et al, *Nat Med*, 2022

Is grade ≥3 toxicity correlated with survival?



HR 0.44, P= 6.06×10^{-5}

Patients who developed serious irAE (N=136) have better survival rates than patients with no serious irAE (N=312). Similar correlation observed in analysis adjusted for sex, age, cancer type and stage.

Summary for part 2

- 8 novel variants reached suggestive significance ($P<5x10^{-6}$) in relation any serious irAE but none reached GWAS significance following an analysis in a small validation cohort.
- rs35429660 is a promising marker linked with serious irAE and OST score. It is a significant eQTL for *ZNF701*
- Markers rs6509652 linked with ≥gr 3 hepatitis (also mapped to *ZNF701),* rs112264014 linked with GI events (mapped to *MAST3*) are organ-specific toxicity variants of interest.
- Association between presence of serious irAE and improved survival is seen but follow up survival data still immature. The best markers will be those that can predict toxicity risk which are not associated with better survival.

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Conclusion and future directions

- Identified potential genetic determinants of capecitabine and ICI induced serious toxicities but further validation is required
- Some markers mapped to candidate genes that could explain the mechanisms behind the toxicity and guide future functional work
- Results of our datasets can contribute to future meta-analyses
- Being able to predict toxicity risk can help treatment planning and implement toxicity surveillance strategies
- Toxicity profile determined by these markers will need to be considered alongside markers of efficacy to maximise treatment benefit while minimizing serious toxicity

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