GENDER·NET Plus
Promoting gender equality in H2020 and the ERA

Final Dissemination Conference

13-14 February 2023

G-DEFINER

Gender Difference in sidE eFfects of ImmuNotherapy: a possible clue to optimize cancEr tReatment

Presentation of the research project

WECAN Academy, Bruxelles

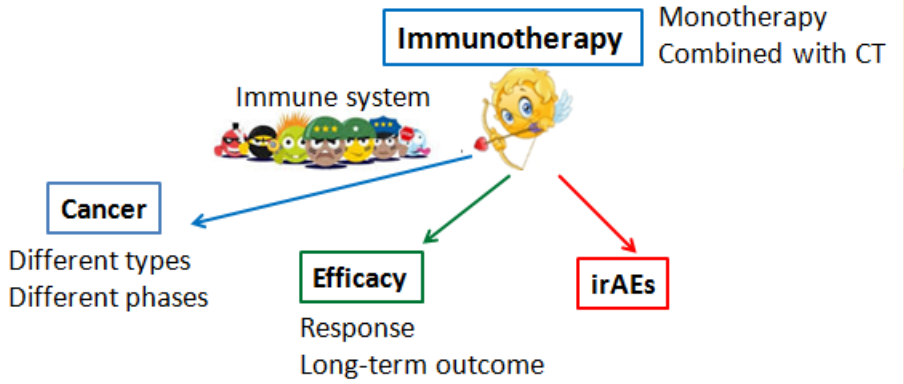
January 25th 2020

The Principal Investigator, Rosalba Miceli, was invited to present G-DEFINER at "WECAN Science 2020" (WECAN Academy, Bruxelles, January 25th 2020). WECAN, is an informal network of leaders of cancer patient umbrella organisations active in Europe.

Partner, meeting, date

Presentation of the research project

Background



- Immune checkpoint inhibitors (ICIs) are immunomodulatory antibodies that
  - increase the cancer patients immune response.
  - significantly improve the oncologic outcomes (PFS, OS).
- ICI are used to treat several cancer types: melanoma, lung and liver cancer, leukemia and lymphoma, urothelial cancers, and subtypes of breast and colon cancer.

- ICI use is associated with unique immune-related adverse events (irAEs), caused by a general activation of the immune system.
  - Main organ systems affected: gastrointestinal tract, endocrine glands, skin, blood (hematologic toxicity), kidney, liver, heart (e.g. myocarditis), nervous system.
  - irAEs G≥2 may cause treatment discontinuation and require treatment.
  - High grade irAEs can occasionally be life threatening.

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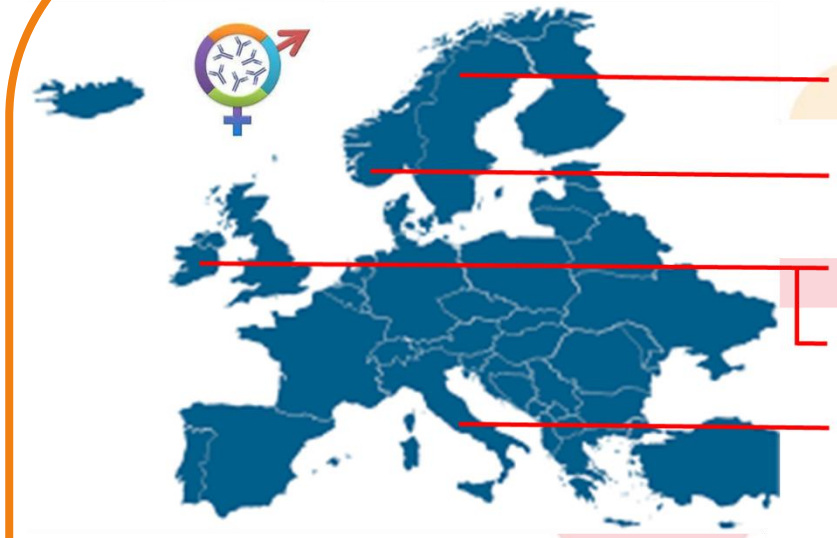
Presentation of the research project

G-DEFINER Consortium



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Main study aim

To investigate the irAEs inequalities between female and male patients (incidence, type, grade) according to different clinical-biological features and intersectional dimensions

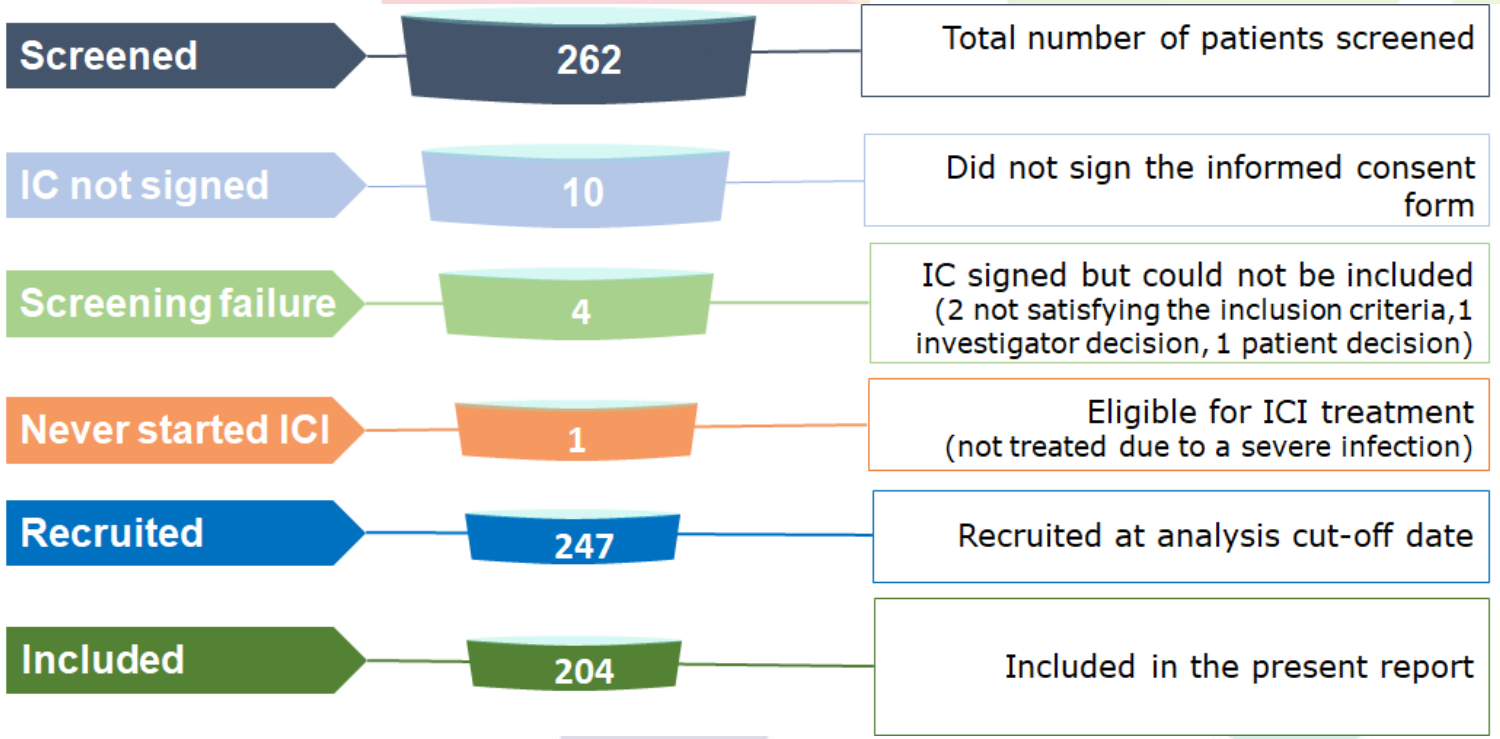
G-DEFINER characteristics

- **Multidisciplinary project:** Medical oncology, biology, biostatistics, bioinformatics.
- **Multicentre observational prospective clinical study:**
  - Clinical protocol ➔ Centers' Ethics Committees approval
  - CRF ➔ eCRF (web database)
  - SOPs (study conduction and biological material collection)

clinicaltrials.gov ID: NCT04435964, ENCePP ID: EUPAS31282, Protocol DOI: 10.5281/zenodo.4142124

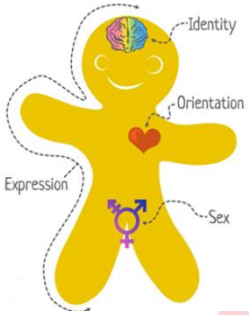
Patients recruitment

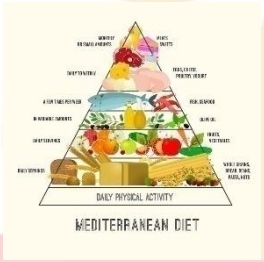
- Started in June 2020 (ITA), closed at 31st January 2023.
  - COVID-19 pandemic impacted on recruitment.
- The present report is an interim analysis at early January 2023.
  - Follow-up data are still maturing and database is being finalized.



Wide breadth of collected data

Baseline





Outpatient visit/medical charts:

- demographics, comorbidities,
- disease and treatment characteristics,
- follow-up.

Times T0 and T1

DNA and RNA

- lab variables and hormones
- immune-related genes
- single-nucleotide polymorphisms
- microbiota
- cytokines

EQ-5D-5L

NCCN National Comprehensive Cancer Network®

Outpatient visits:

- characteristics intersecting with sex&gender norms and roles (mainly psycho-social and behavioral) possibly associated with the study outcome (irAEs):
  - quality of life/distress, diet/eating habits (microbiota interference).
  - personal sense of being, ethnicity, marital status, living arrangement, education, occupation, income, smoking and alcohol habits, physical activity level.
- Lab variables, gene expression, SNPs, microbiota, cytokines.

Key features significantly different between F and M populations

	Overall	F	M	p
N. (%) of patients	**204**	**86**	**118**	
Disorders thyroid gland	13 (6.4)	**8 (9.3)**	**5 (4.2)**	**0.158**
Mental/behavioural disorders	12 (5.9)	**8 (9.3)**	**4 (3.4)**	**0.141**
Diseases nervous system	12 (5.9)	**2 (2.3)**	**10 (8.5)**	**0.129**
Diseases eye/ear	10 (4.9)	**6 (7.0)**	**4 (3.4)**	**0.077**
Hypertensive diseases	77 (37.7)	**38 (44.2)**	**39 (33.1)**	**0.111**
Other heart diseases	29 (14.2)	10 (11.6)	19 (16.1)	0.421
Genitourinary diseases	10 (4.9)	**0 (0.0)**	**10 (8.5)**	**0.006**
Cancer type				0.838
CR MSI high	12 (5.9)	7 (8.1)	5 (4.2)	
Headneck	26 (12.7)	11 (12.8)	15 (12.7)	
Lung	86 (42.2)	36 (41.9)	50 (42.4)	
Melanoma	74 (36.3)	30 (34.9)	44 (37.3)	
Urogenital (included renal)	6 (2.9)	2 (2.3)	4 (3.4)	
ICI setting				**0.012**
Neoadj/adj	32 (15.7)	9(10.5)	23 (19.5)	
Neoadjuvant	2 (1.0)	**0 (0.0)**	**2 (1.7)**	
Adjuvant	30 (14.7)	**9 (10.5)**	**21 (17.8)**	
Advanced 1st	123 (60.3)	**57(66.3)**	**66 (55.9)**	
Advanced NA	6 (2.9)	6 (7.0)	0 (0.0)	
Advanced 1st	117 (57.4)	51 (59.3)	66 (55.9)	
Advanced≥2nd,maintenance	49 (24.0)	20 (23.3)	29 (24.6)	
Advanced 2nd	30 (14.7)	12 (14.0)	18 (15.3)	
Advanced >2nd	14 (6.9)	**4 (4.7)**	**10 (8.5)**	
Maintenance	5 (2.5)	**4 (4.7)**	**1 (0.8)**	
Causes of ICI interruption during the study				**0.099**
No interruption	132 (64.7)	**48 (55.8)**	**84 (71.2)**	
ICI completed	1 (0.5)	0 (0.0)	1 (0.8)	
CR	4 (2.0)	2 (2.3)	2 (1.7)	
irAE w/wo other cause		**23 (26.8)**	**14 (11.0)**	
irAE	31 (15.2)	**19 (22.1)**	**12 (10.2)**	
CR+irAE	2 (1.0)	**1 (1.2)**	**1 (0.8)**	
irAE+Relapse/PD	4 (2.0)	**3 (3.5)**	**1 (0.8)**	
Relapse/PD	24 (11.8)	12 (14.0)	12 (10.2)	
Other causes	3 (1.5)	0 (0.0)	3 (2.5)	
Death	3 (1.5)	1 (1.2)	2 (1.7)	

Key features significantly different between F and M populations

	Overall	F	M	p
N. (%) of patients	**204**	**86**	**118**	
Personal sense of being				
Woman	82 (40.2)	82 (95.3)	0 (0.0)	--
Man	116 (56.9)	2 (2.3)	114 (96.6)	
Not reported	6 (2.9)	2 (2.3)	4 (3.4)	
Marital status				**0.005**
Couple	123 (60.3)	**43 (50.0)**	**80 (67.8)**	
Not couple	57 (27.9)	30 (34.9)	27 (22.9)	
Divorced/Separated	18 (8.8)	8 (9.3)	10 (8.5)	
Never married	18 (8.8)	6 (7.0)	12 (10.2)	
Widowed	21 (10.3)	**16 (18.6)**	**5 (4.2)**	
Not reported	24 (11.8)	13 (15.1)	11 (9.3)	
Living arrangement				0.156
Alone	42 (20.6)	**22 (25.6)**	**20 (16.9)**	
Not alone	140 (68.6)	53 (61.6)	87 (73.7)	
Children	11 (5.4)	7 (8.1)	4 (3.4)	
Other	3 (1.5)	1 (1.2)	2 (1.7)	
Parents	3 (1.5)	1 (1.2)	2 (1.7)	
Parents+sibl	1 (0.5)	1 (1.2)	0 (0.0)	
Partner	90 (44.1)	**29 (33.7)**	**61 (51.7)**	
Partner+children	32 (15.7)	14 (16.3)	18 (15.3)	
Not reported	22 (10.8)	11 (12.8)	11 (9.3)	
Income				0.220
≤2000	18 (8.8)	9 (10.5)	9 (7.6)	
>2000, ≤3000	50 (24.5)	21 (24.4)	29 (24.6)	
>3000, ≤5000	37 (18.1)	14 (16.3)	23 (19.5)	
>5000	53 (26.0)	**17 (19.8)**	**36 (30.5)**	
Not reported	46 (22.5)	25 (29.1)	21 (17.8)	
BMI				**0.009**
Underweight	10 (5.2)	**9 (11.5)**	**1 (0.9)**	
Healthy Weight	86 (44.8)	32 (41.0)	54 (47.4)	
Overweight	72 (37.5)	**26 (33.3)**	**46 (40.4)**	
Obesity	24 (12.5)	11 (14.1)	13 (11.4)	
W/H not reported	12	8	5	
Mediterranean diet score				0.052
Mean (SD)	5.95 (2.08)	6.31 (2.12)	5.69 (2.01)	
Not reported	30 (14.7)	13 (15.1)	17 (14.4)	
Physical activity				0.218
Inactive	19 (9.3)	9 (10.5)	10 (8.5)	
Very low int.	76 (37.3)	**35 (40.7)**	**41 (34.7)**	
Low int.	57 (27.9)	20 (23.3)	37 (31.4)	
Moderate int.	30 (14.7)	14 (16.3)	16 (13.6)	
High int.	6 (2.9)	0 (0.0)	6 (5.1)	
Not reported	16 (7.8)	8 (9.3)	8 (6.8)	

Expected correlation between living arrangement and marital status

	Overall	Not couple	Couple	Not reported	p
N. (%) of patients	**204**	**57**	**123**	**24**	
Living arrangement					<0.001
Alone	42 (20.6)	37 (64.9)	0 (0.0)	5 (20.8)	
Children|parents|other	18 (8.8)	14 (24.6)	1 (0.8)	3 (12.5)	
Partner+-children	122 (59.8)	1 (1.8)	**119 (96.7)**	2 (8.3)	
Not reported	22 (10.8)	5 (8.8)	3 (2.4)	14 (58.3)	

Key QoL features significantly different between F and M populations

Dimensions: health today, Self care (washing/dressing), Usual activities, Pain/discomfort, Anxiety/depression

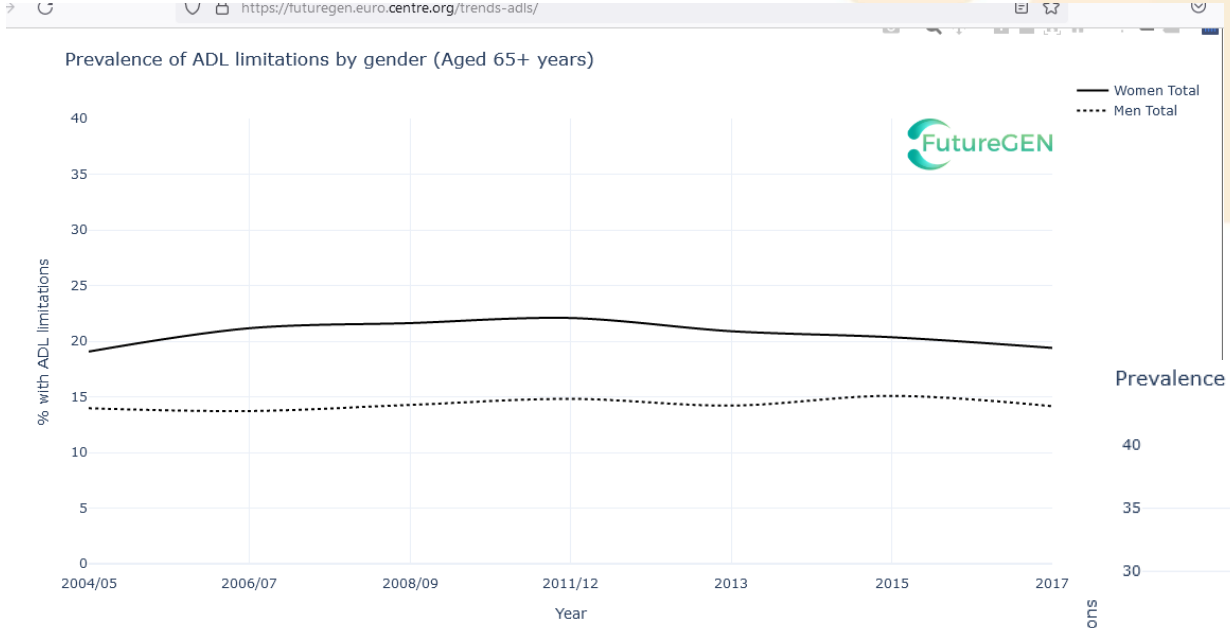
Dimensions: Distress thermometer; Problems: Practical, Family, Emotional, Spiritual/religious, Physical.

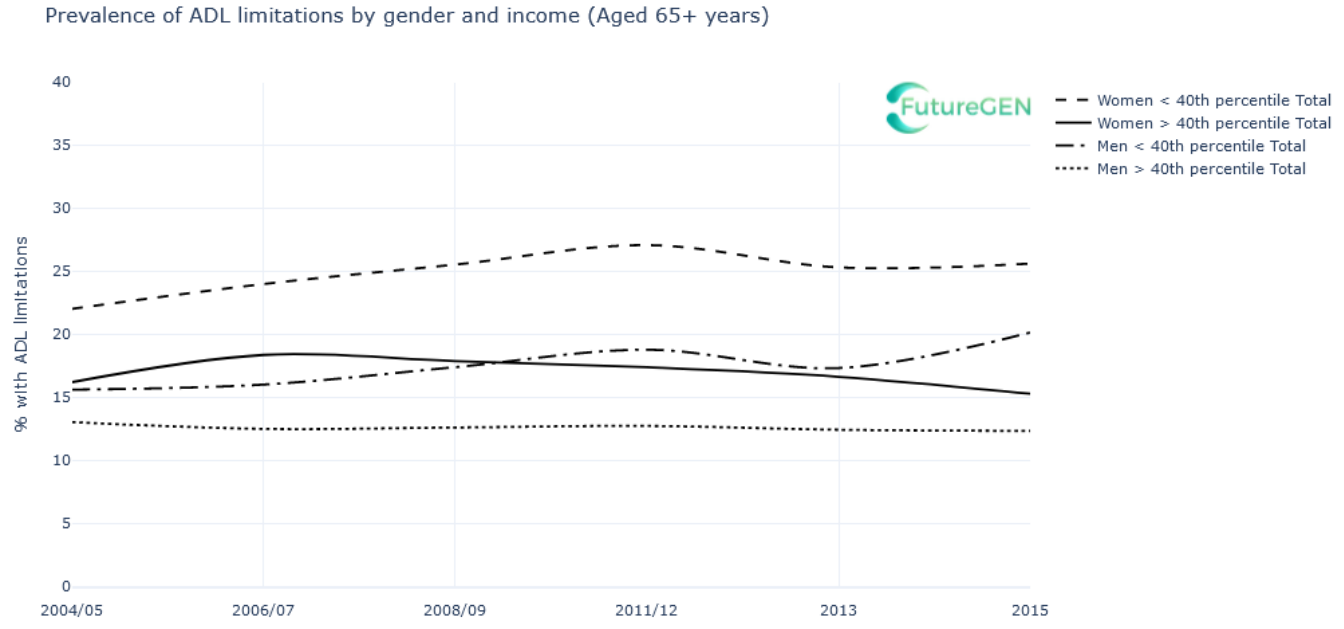
	Overall	F	M	p
N. (%) of patients	**179**	**75**	**104**	
NCCN emotional problems:				
Fear	36 (20.1)	22 (29.3)	14 (13.5)	0.015
Loss of interest in usual activities	33 (18.4)	21 (28.0)	12 (11.5)	0.009
Sexual	20 (11.2)	1 (1.3)	19 (18.3)	0.001
Genitourinary diseases	10 (4.9)	0 (0.0)	10 (8.5)	0.006
EQ-5D-5L				
Anxiety/depression (mean (SD))	1.82 (0.87)	2.00 (0.90)	1.69 (0.83)	0.019
Pain/discomfort (mean (SD))	1.94 (0.99)	2.15 (1.14)	1.80 (0.85)	0.020

"Sex balancing weights": the most important baseline characteristics able to discriminate F and M patients were selected by applying a logistic regression with Least Absolute Shrinkage Selection Operator (LASSO) penalty function. Disease and treatment characteristics were not included in the model.

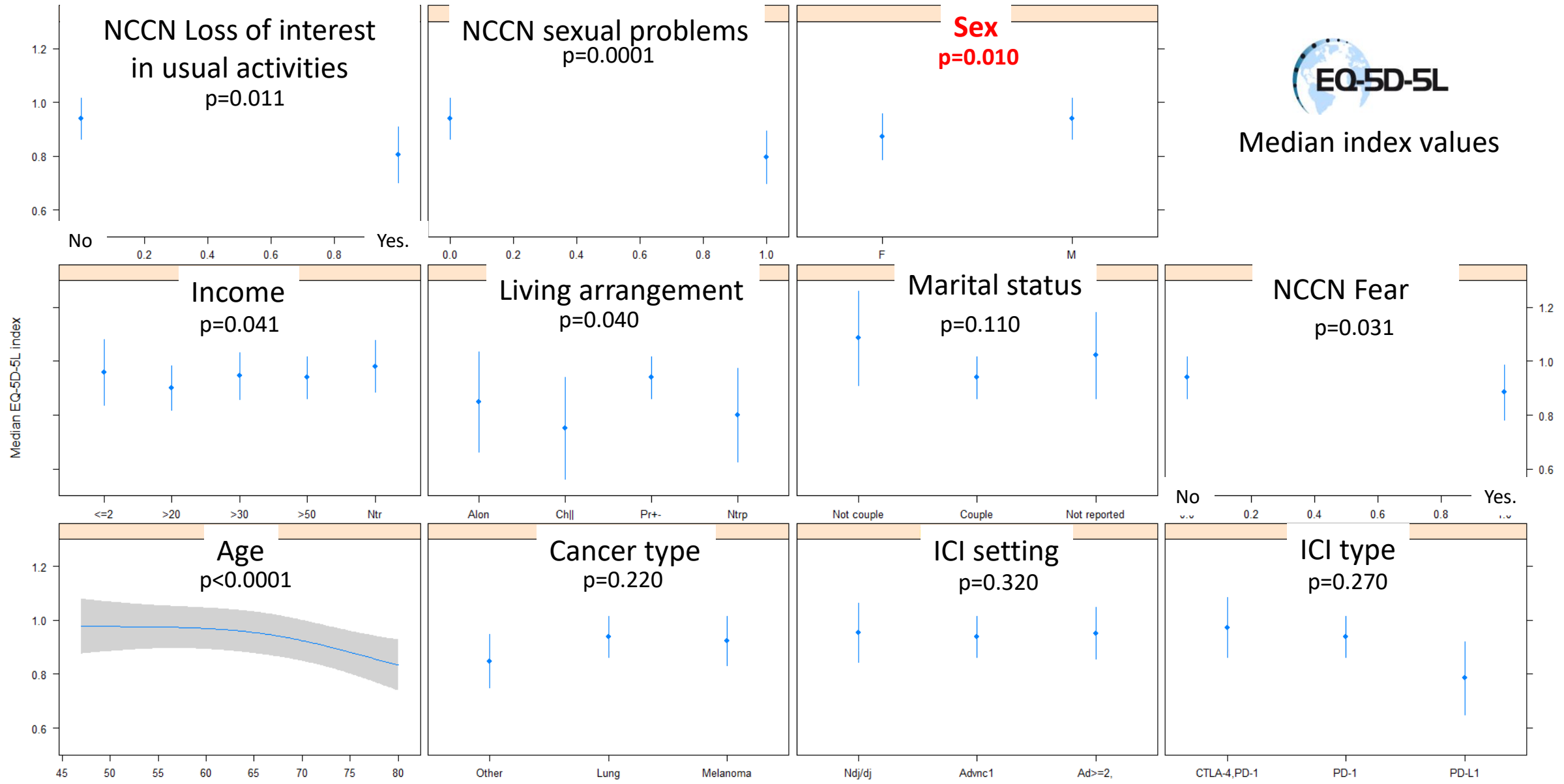
FutureGen Data Navigator findings

Activities of Daily Living (ADLs)

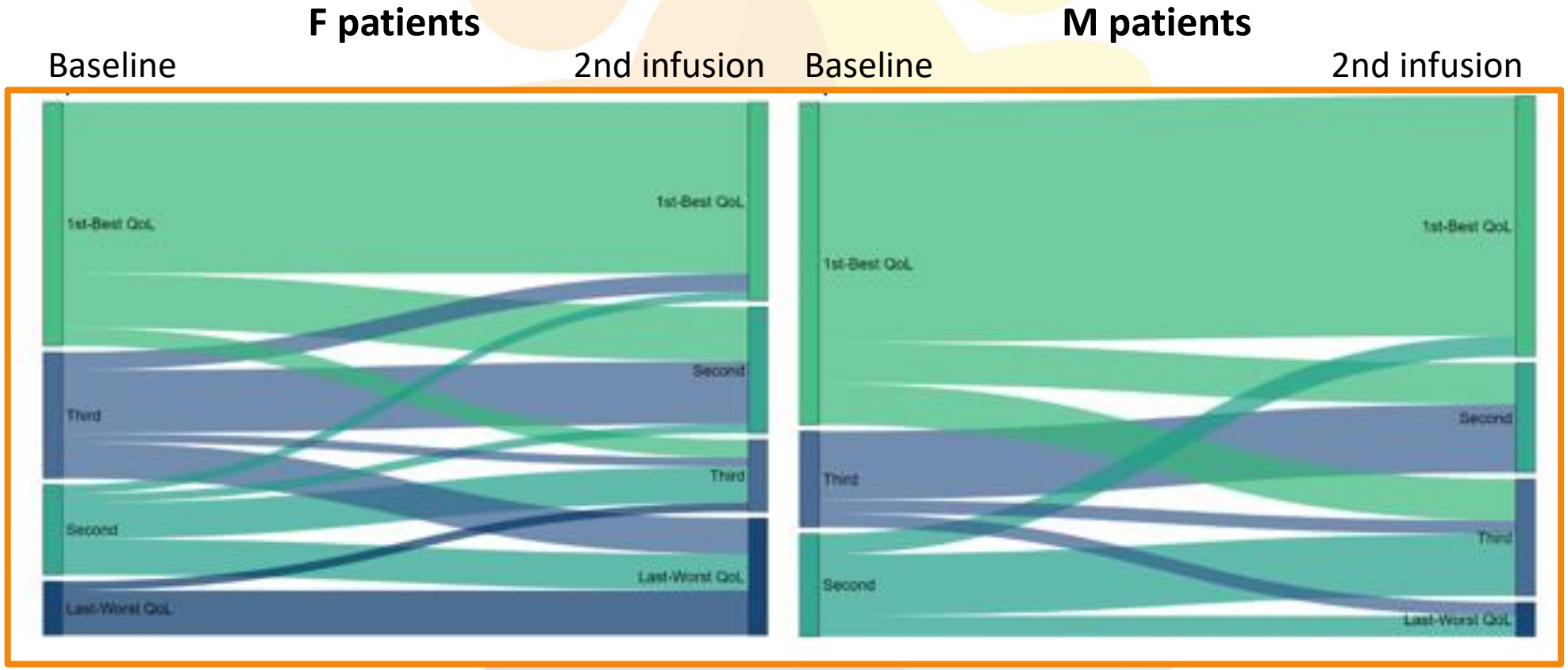




Sex is independently associated with EQ-5D-5L QoL index at multivariable analysis



Disease can decrease patients' QoL more in F patients than M patients

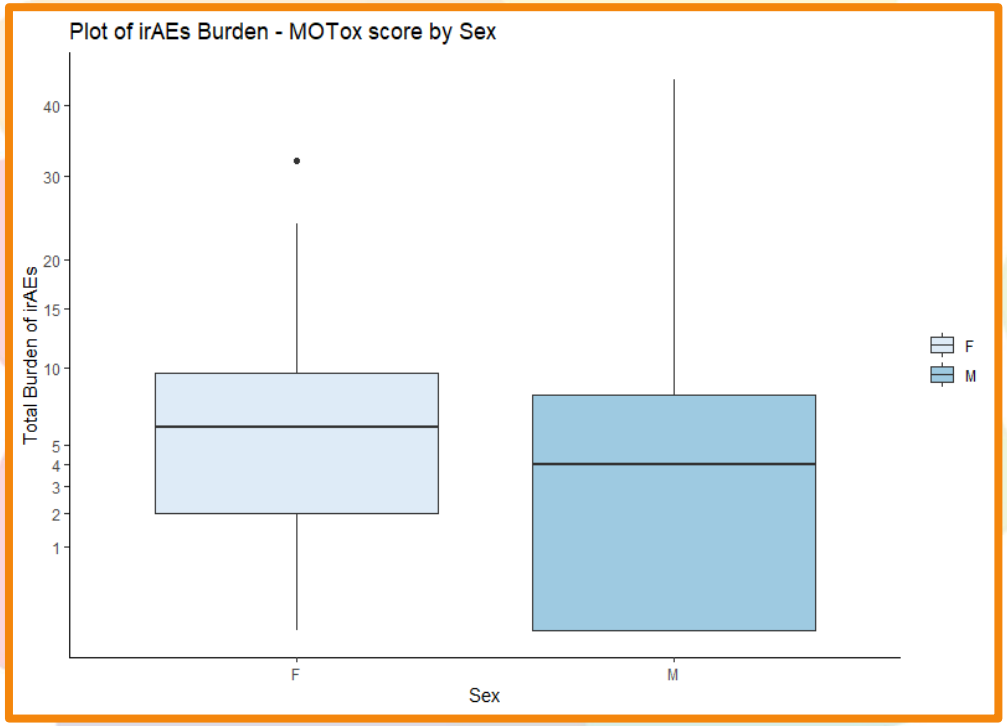


Cluster analysis was applied to find groups of patients with similar QoL characteristics, independently of sex, separately for 1st and 2nd infusion data. The clusters were ordered (from best QoL to worse QoL) according to the QoL features values.

Main study outcome: F patients have significantly higher irAEs burden than M patients

BURDEN OF IRAES

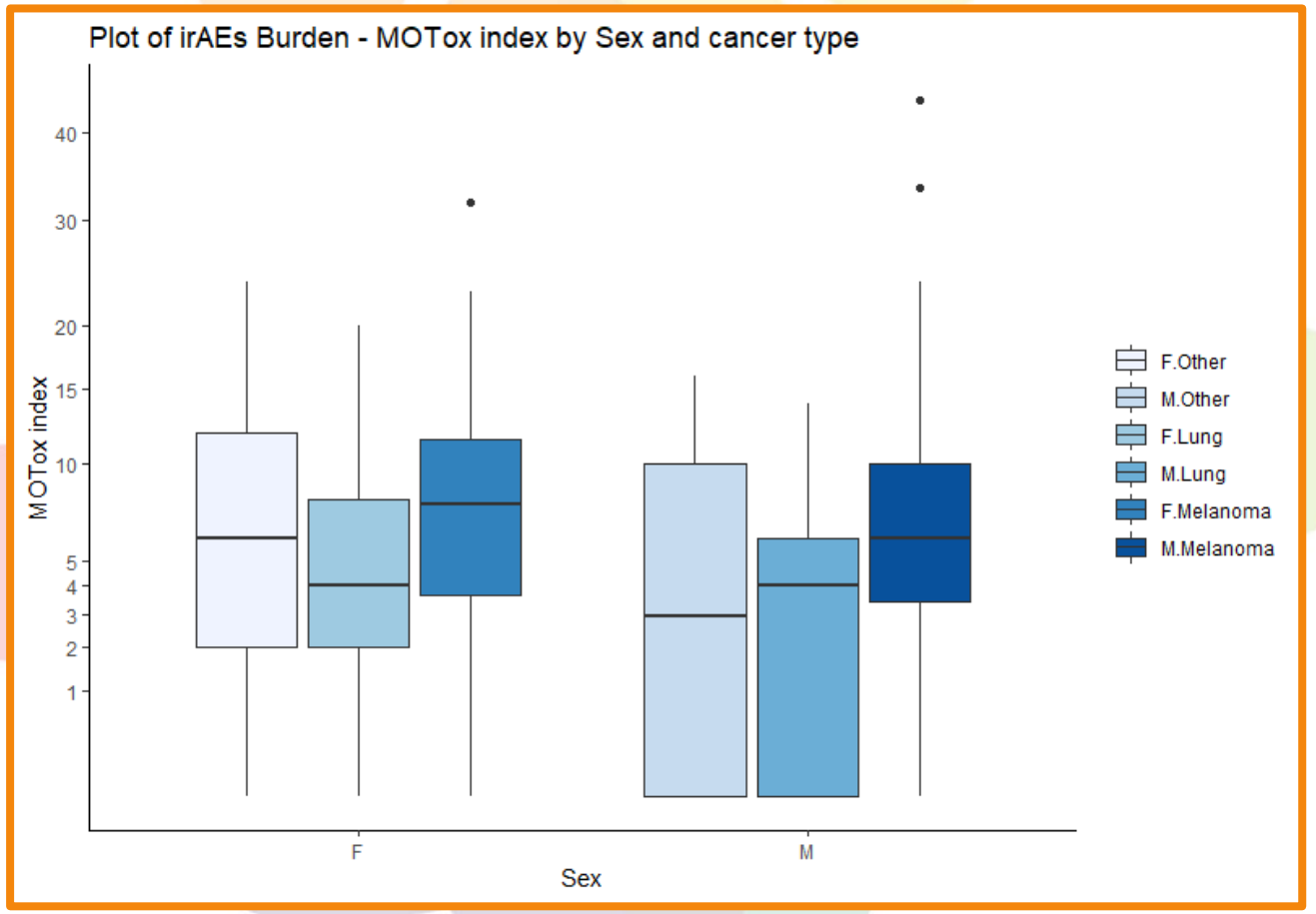
$$MOTox_i^k = average\ toxic\ level_i^k + worst\ grade_i^k,$$



The difference between the medians was statistically significant at **univariable quantile regression analysis** (**p=0.024**).

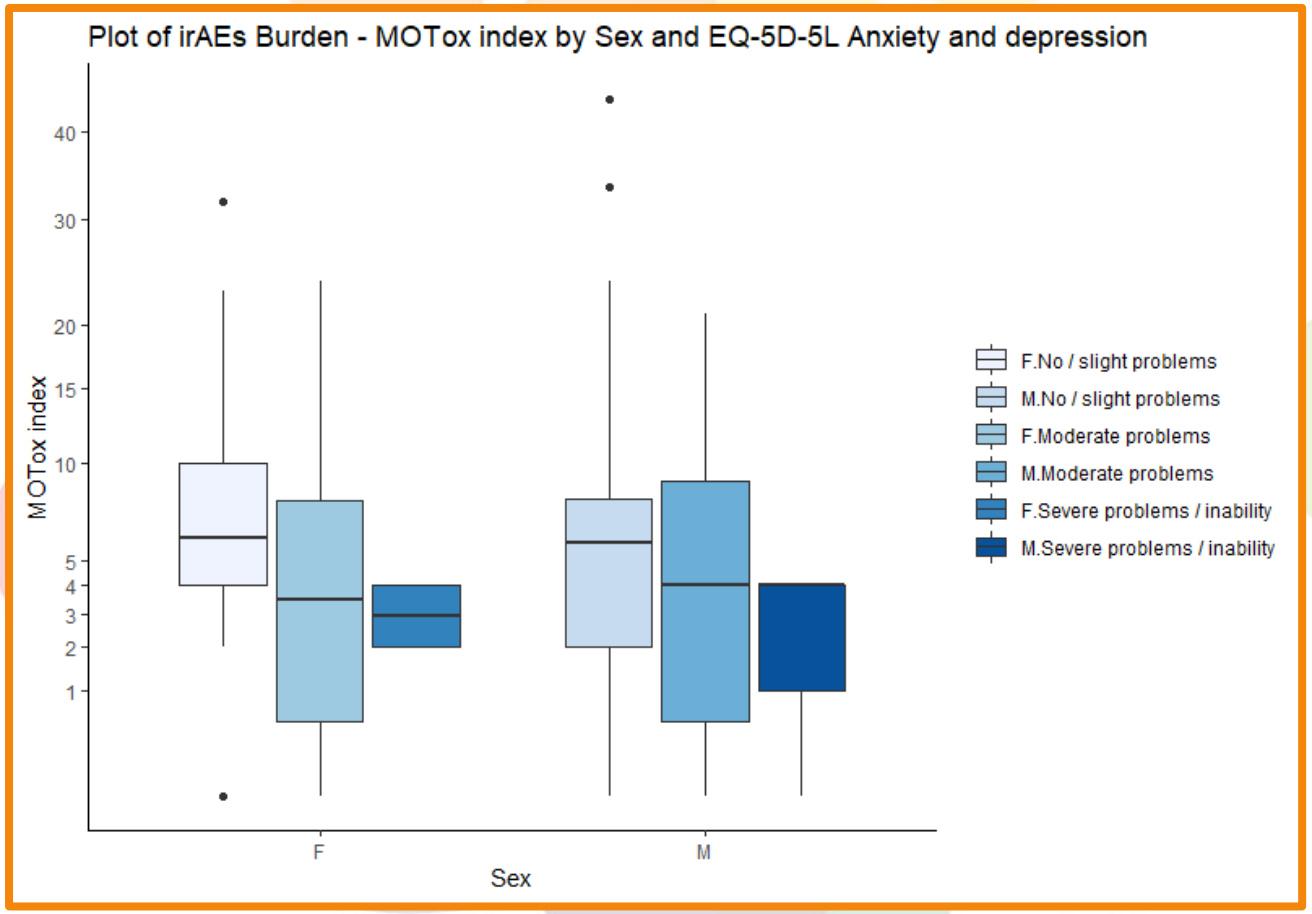
In the **multivariable quantile regression model**, including cancer type, ICI type and setting as adjustment covariates, using as weights the "sex balancing weights", the difference between the medians, 1.8, was statistically significant (**p=0.048**).

Main study outcome: interaction between sex and cancer type



irAE burden was higher in F than M for patients with melanoma or with tumors other than melanoma and lung (p-value = 0.019)

Main study outcome: interaction between sex and EQ-5D-5L anxiety/depression

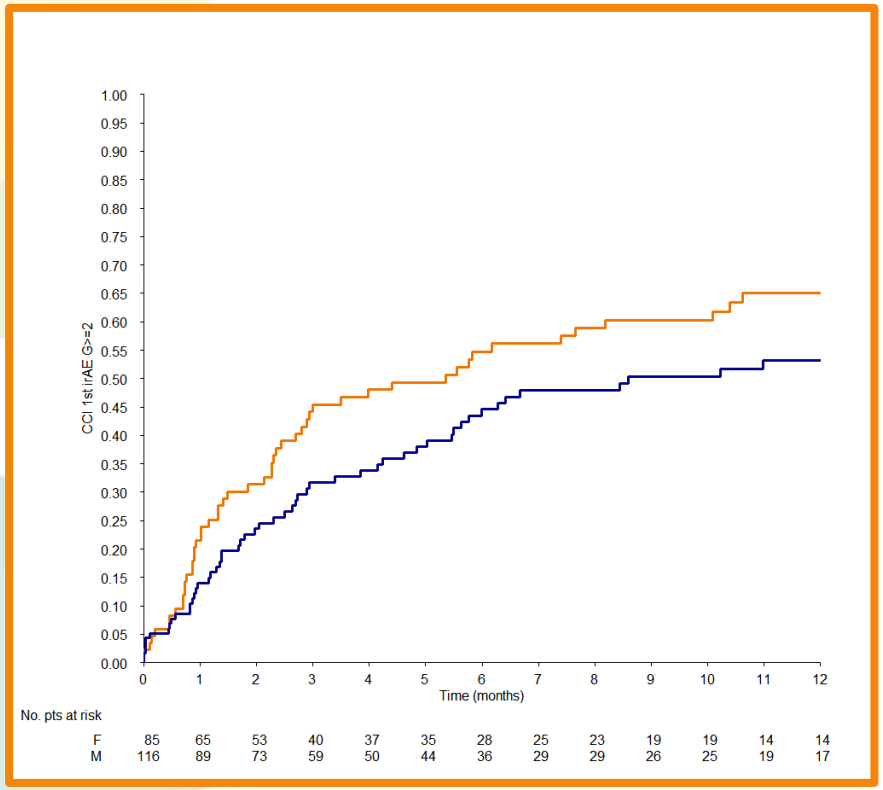


For both F and M there was an inverse association between the irAS burden and the anxiety/depression intensity: the patients with no or slight problems (in particular F) had the highest burden (p=0.148).

Main study outcome: F patients have higher incidence of first G≥2 irAE than M patients

N. (%) of patients	F (N=86) n. irAEs	F (N=86) n. patients	M (N=118) n. irAEs	M (N=118) n. patients	p value
Number of irAEs per patient					0.465
No events	--	15/86 (17.4%)	--	34/118 (28.8%)	
1 irAE	--	22/86 (25.6%)	--	27/118 (22.9%)	
2 irAEs	--	15/86 (17.4%)	--	20/118 (17.0%)	
3 irAEs	--	14/86 (16.3%)	--	12/118 (10.2%)	
4 irAEs	--	9/86 (10.5%)	--	13/118 (11.2%)	
≥5 irAEs	--	11/86 (12.8%)	--	12/118 (10.2%)	
Any grade adverse event	204	71/86 (82.6%)	240	84/118 (71.2%)	**0.069**
Grade 1	108	55/86 (64.0%)	144	63/118 (53.4%)	0.152
Grade 2	64	42/86 (48.8%)	69	47/118 (39.8%)	0.253
Grade 3	27	21/86 (24.4%)	22	20/118 (17.0%)	0.217
Grade 4	5	5/86 (5.8%)	3	2/118 (1.7%)	0.135
Grade 5	0	0/86 (0.0%)	2	2/118 (1.7%)	0.510
First irAE G≥2	53	53/86 (61.6%)	57	57/118 (48.3%)	0.073*
First irAE G≥3	24	24/86 (27.9%)	21	21/118 (17.8%)	0.106*
System Organ Class					
Systemic	25	20/86 (23.3%)	20	19/118 (16.1%)	0.212
Dermatologic	35	21/86 (24.4%)	43	29/118 (24.6%)	1
Rheumatic	10	8/86 (9.3%)	24	17/118 (14.4%)	0.291
Gastrointestinal	35	24/86 (27.9%)	48	27/118 (22.9%)	0.419
Opthalmic	2	1/86 (1.2%)	2	2/118 (1.7%)	1
Neurologic	4	2/86 (2.3%)	4	2/118 (1.7%)	1
Cardiac	3	3/86 (3.5%)	4	4/118 (3.4%)	1
Endocrine	45	31/86 (36.1%)	35	24/118 (20.4%)	**0.016**
Renal	2	2/86 (2.3%)	7	6/118 (5.1%)	0.472
Hepatic	20	17/86 (19.8%)	20	11/118 (9.3%)	**0.040**
Respiratory	19	11/86 (12.8%)	24	17/118 (14.4%)	0.838
Hematologic	4	4/86 (4.7%)	9	8/118 (6.8%)	0.765

Crude cumulative incidence of first G≥2 irAE



6-month: F: 54.8% (45.7-67.1%), M: 44.6% (35.8-55.5%).
12-month: F: 65.0% (54.9-77.0%), M: 53.3% (43.9-64.6%).
(p=0.073).
At the multivariable analysis (applying the "sex balancing weights" and with adjustment for cancer type, ICI type, and ICI setting) the sHR was 1.33 (0.91-1.93) (p=0.140).

Collection and biological analysis of patient samples

Biological Analysis Methods

Design: Multicentre observational prospective clinical study

clinicaltrials.gov ID: NCT04435964, ENCePP ID: EUPAS31282, Protocol DOI: 10.5281/zenodo.4142124

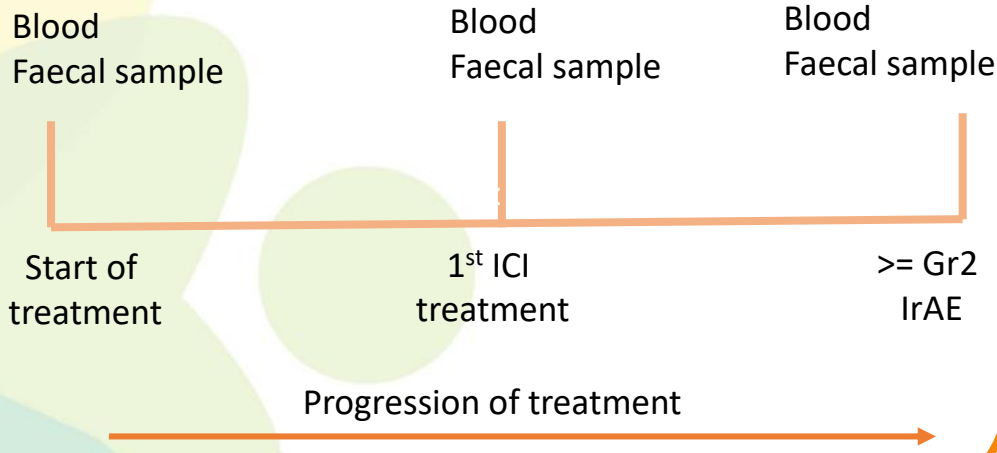
Data:

Times T0 and T1

- lab variables and hormones
- immune-related genes (Blood)
- single-nucleotide polymorphisms (Blood)
- microbiota (Faecal)

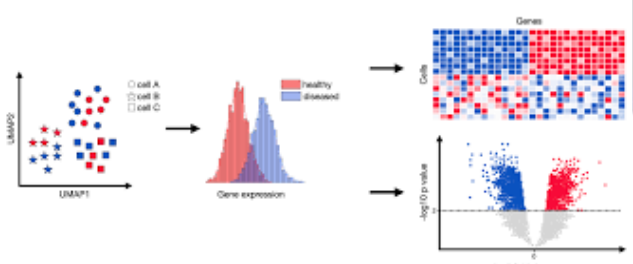
DNA and RNA

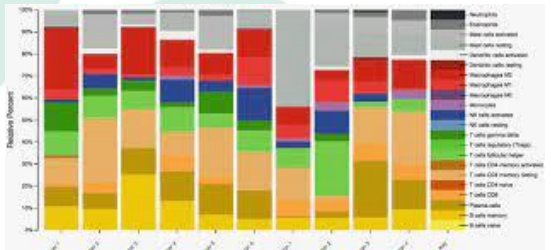
Collection of Biological Samples

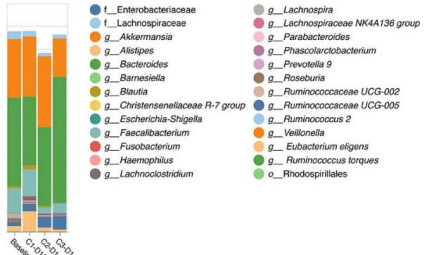


Time Point	Patient samples
T0 + T1	214 patients
T2	42 patients

- **Lab variables and hormones** – Assessment of inflammation as biomarker of future IrAEs
- **Gene Expression Analysis** – In Silico analysis of patient's immune contexture with IrAEs
- **Single-nucleotide polymorphisms** - Correlation of patient's genetic background with IrAEs
- **Microbiota** - to study microbial communities found in and on the human body. The goal of human microbiome profiling studies is to understand their role in health and disease.







Biological analyses on blood specimens: Gene expression profiling

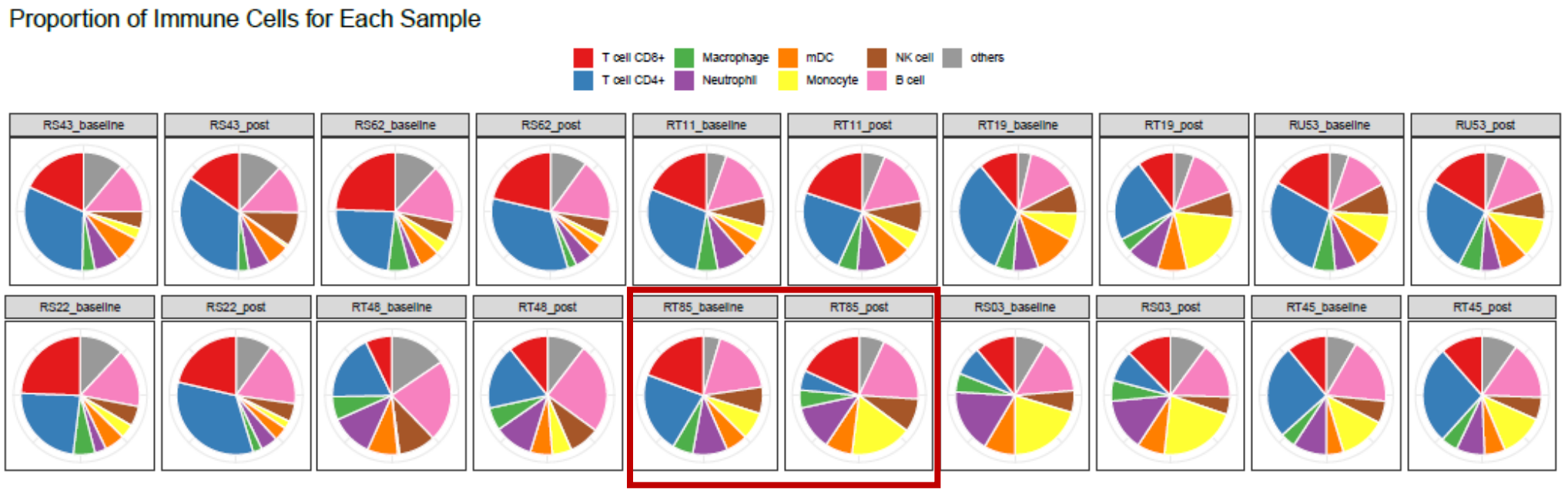
Gene expression profiling for immune assessment

i) to determine the relationship of gene expression with irAEs

ii) Use In Silico approaches to correlate the immune contexture of patients blood with irAE occurrance;

iii) to explore biological oriented immune signatures/pathways (i.e. IFN-γ);;

Methods: i) whole transcriptome profiling by RNAseq; ii) deconvolution bioinformatics analysis of immune cellular subsets; iii) imputation of immune-related signatures (hacksig R package; https://github.com/Acare/hacksig).



Future plans

- Database: follow-up updating and finalization
- Deepen the analysis of association between irAEs (burden and incidence) and sex and psycho-social / QoL features.
- Biological data analyses:
  - Routine lab biomarkers (e.g. monocytes, lymphocytes, platelets etc)
  - Blood samples: gene expression, SNPs, cytokines;
  - Stool samples: microbiota;
  - search for inflammatory markers and signatures.
- irAE predictive models.

Expected socio-economic impact

- **The results presented so far highlighted the importance of applying an intersectional approach** to:
  - Discover irAEs inequalities between female and male patients
  - Discover more frail subgroups to be monitored more strictly (e.g. anxious/fearful)
  - Characterize patients at higher irAEs risk:
    - allow timely diagnosis and personalization of irAEs treatment approaches
    - reduce ICI interruptions (especially for F patients) and maximize ICI efficacy.

- **The results, obtained in a real world setting (outside clinical trials), will more easily be translated in clinical setting.**

- **This ultimately contributes to:**
  - **Equitable medical treatment,** ensuring that interventions are inclusive and effective for all individuals.
  - **Reduce healthcare system costs**.

Directions for future research

- Design prospective clinical studies to evaluate the effectiveness of interventions directed to reduce health inequalities (irAEs, therapy/interventions efficacy) between F and M patients (e.g. effectiveness implementation design).
- Implement personalized approaches to disease prevention and treatment, taking into account, together with disease characteristics, also individual characteristics (psycho-socio-economical features and quality of life).
- Carry out patients and public participatory research (patients and the public work in partnership with researchers).
- These research lines could provide policy makers with information useful to implement concrete gender medicine actions.

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Patients and their families

Thank you for your attention