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TET2 SNPs and the Risk of Cancer in the START, SMART and ESPRIT Cohorts

Daniel D. Murray[1], Birgit Grund[2], Cameron MacPherson[1], Christina Ekenberg[1], Adrian Zucco[1], Joanne Reekie[1], Dahlene Fusco[3], Julien Gras[4], Jan Gerstoft[5], Marie Helleberg[1], Alvaro Borges[1], Mark Polizzotto[6], Jens D Lundgren[1] for the INSIGHT START, SMART and ESPRIT study groups.

[1]Centre of Excellence for Health, Immunity and Infections (CHIP), Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.[2] School of Statistics, University of Minnesota, Minneapolis, Minnesota, USA. [3]Tulane University Medical Center, Tulane University, New Orleans, Louisana, USA [4] Service de Maladies infectieuses et tropicales, APHP-Hôpital Saint Louis, Paris, France. [5]Rigshospitalet, Copenhagen, Denmark. [6]The Kirby Institute for Infection and Immunity in Society, UNSW Sydney, Sydney, New South Wales, Australia

BACKGROUND

- The Ten-eleven methylcyosine dioxygenase 2 gene (TET2) encodes a protein that is involved in locus-specific DNA de-methylation of previously methylated and silenced genes through the oxidation of 5-methylcytosine (5mC)
- The primary focus of the TET2 literature is its role in cancer, with loss of TET2 function implicated in the development of both hematological malignancies and solid tumours (1)
- However, there is also a developing literature associating this gene with HIV replication (2), as well as retroviral associated malignancy (3-4)
- Previously, we identified two groups of SNPs in the TET2 gene, one group associated with higher and the other with lower HIV Viral Load (VL) (5)
- These results indicated that TET2 is involved in HIV replication and that the identified SNPs impacts the genes function
- As TET2 also plays a role in cancer development, we hypothesized that the HIV-VL associated TET2 SNPs would also impact the risk of cancer
- Therefore, in this study, we performed a targeted association analysis between VL-associated TET2 SNPs and risk of cancer across START, SMART and ESPRIT studies

METHODS

- We analysed SNP and clinical data from three trials conducted by the INSIGHT clinical trials network, the START, SMART and ESPRIT studies.
- Participants were genotyped using a custom content Affymetrix Axiom SNP array, consisting of 770,558 probes, enriched with markers related to immune dysfunction
- 36 SNPs from the TET2 gene were analyzed, selected because they were associated with HIV-VL in a previous study (5).
- Cancer includes any malignancy diagnosed during study follow-up, except non-melanoma skin cancer. Cancers were reported with date of diagnosis and adjudicated by an independent endpoint review committee using standardized criteria across the clinical trials.
- The association of each SNP with cancer was estimated using Cox proportional hazards regression models. Models were fitted separately in each study, with study arm, gender, age at study entry and the first four eigenvectors (to control for population structure) included as co-variates. Only SNPs with minor allele frequency (MAF) > 1% were analyzed.
- P-values for associations with clinical outcomes are shown uncorrected and also adjusted for multiple comparisons (Ap) using the max(T) permutation test (10000 permutations)
- As SNPs were mainly present in persons of Black race, we performed a sensitivity analysis for significantly associated SNPs restricted to the Black participants of START and SMART

RESULTS

Table 1. Study Entry Characteristics for the START, SMART and ESPRIT cohorts analysed in this study

	START (n=2546[1])	SMART (n=2283[1])	ESPRIT (n=2891[1])
Age (years) Median (IQR)	36 (29, 45)	44 (38, 50)	40 (34, 46)
Female (%)	20	26	18
Race (self-reported)			
Asian (%)	1	1	12
Black (%)	23	38	9
White/other (%)	76	60	79
CD4+ count (cells/mm^3) Median (IQR)	651 (585, 759)	572 (455, 773)	451 (368, 582)
HIV RNA level (log10 copies/mL) Median (IQR)	4.17 (3.54, 4.66)	2.60 (1.70, 3.45)	1.70 (1.70, 2.60)
CD8+ T cell count (cells/mm^3) Median (IQR)	1,062 (790, 1,431)	n/a	n/a
Nadir CD4+ T cell count (cells/mm^3) Median (IQR)	545 (473, 642)	n/a	n/a
On ART at study entry (%)	0	79.5	100
ART naïve (%)	100	6.0	0
Geographical region			
U.S.[2] (%)	18	80.4	25
Europe/Australia/Israel[2] (%)	49	11.2	49
South America/Mexico[2] (%)	20	5.5	14
Asia[2] (%)	0	0.4	11
Africa[2] (%)	13	2.5	1

[1]participants who provided specific consent for collecting and analyzing genetic data
[2]Country/Region of residence

- One SNP in TET2, rs6811468, was associated with increased risk of cancer in the START and SMART studies
- The cancers associated with rs6811468 are primarily solid tumours with lung (n=3, 30%) and prostate (n=4, 40%) the most common cancer sites

RESULTS (continued)

- 33, 36 and 20 of the 36 SNPs had MAF>1% and were analyzed for associations with cancer in the START, SMART and ESPRIT studies, respectively.
- In START, 38 participants (pts) (1.5%) were diagnosed with cancer during follow-up. One SNP, rs6811468 (HR=4.50, CI=1.14-17.76, p=0.03, Ap=0.19), was associated with risk of cancer (Figures 1 and 2); in pts with 0, 1 and 2 risk alleles of rs6811468, the incidence of cancer was 35/2412 (1.5%), 3/109 (2.8%) and 0/2 (0%), respectively.
- In SMART, 60 (2.6%) pts were diagnosed with cancer during follow-up. Two SNPs, rs6811468 (HR=2.79, CI=1.41-5.53, p=0.003, Ap=0.03) and rs72955158 (HR=3.24, CI=1.29-8.11 p=0.012, Ap=0.09) were associated with risk of cancer (Figure 1 and 2); pts with 0, 1 and 2 risk alleles of rs6811468, the incidence of cancer was 52/2141 (2.4%), 6/125 (4.8%) and 2/4 (50%), respectively. All 6 cancers associated with rs72955158 in SMART occurred in pts who also had rs6811468.
- In ESPRIT, a total of 110 pts were diagnosed with cancer. No SNPs were significantly associated with cancer in ESPRIT (Figure 1).
- Rs72955158 in START and both rs6811468 and rs72955158 in ESPRIT had MAF <1% and were not assessed in these cohorts.
- Rs6811468-associated cancers were primarily solid tumors (10/11) and occurred in persons of black race (10/11). Specific solid tumour sites include, lung (3/11), prostate (4/11), anal (1/11), cervical (1/11) and skin (1/11). The one non-solid tumour was a non-Hodgkin's lymphoma
- For the sensitivity analysis restricted to pts of black race, the associations between rs6811468 and cancer was assessed in 578 pts in START and 876 in SMART. Although there was a reduction in power, the results of the sensitivity are consistent with those of the main study for both START (HR = 2.88, CI=0.35-20.35, p=0.3) and SMART (HR=2.91, CI=1.46-5.81, p=0.002)

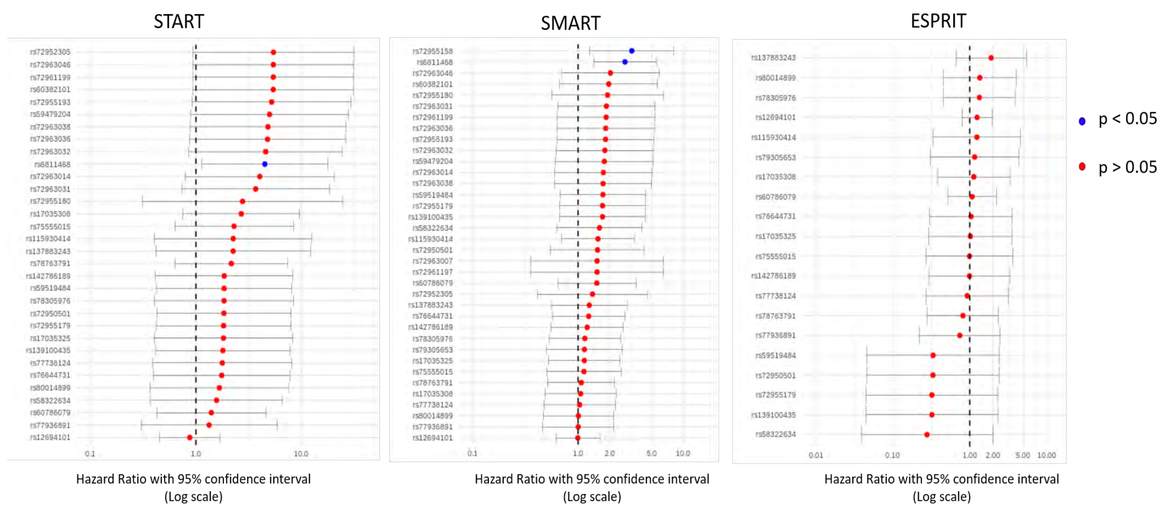

Figure 1. Associations of selected TET2 SNPs with Cancer (any) outcomes in the START, SMART and ESPRIT trials. Only SNPs associated with HIV-VL were included in the analysis. A) Hazard ratios for SNPs associated with cancer (any) after adjusting for age, sex and the first four eigenvectors. SNPs with indeterminate confidence intervals are not presented Remaining SNPs are ordered by hazard ratio (high to low) and plotted using a forest plot. One SNP (rs6811468) was significantly (p=0.045) associated with the cancer outcome after adjusting for all co-variates in the START trial. Two SNPs (rs6811468 and rs72955158) were significantly (p=0.003 and p=0.013 respectively) associated with the cancer outcome after adjusting for all co-variates in the SMART trial. No SNPs were significantly associated with cancer in the ESPRIT trial.

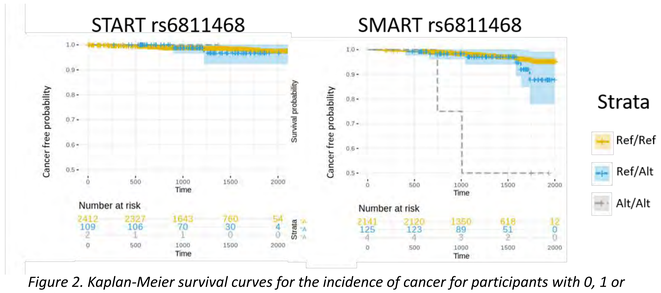

Figure 2. Kaplan-Meier survival curves for the incidence of cancer for participants with 0, 1 or 2 alleles of rs6811468, with 95% CIs. Time is measured in days.

CONCLUSIONS

- rs6811468 was associated with an elevated risk of cancer in both the START and SMART trials
- After multiple testing correction, rs6811468 was significant only in SMART. However, the low number of cancer events in START reduced the power to detect significant associations (START was a much younger cohort with less follow-up compared to SMART)
- In the previous study, rs6811468 was associated with a higher HIV-VL and it is not clear whether the elevated cancer risk is due to a direct impact of the SNPs on the role of TET2 in cancer pathogenesis, or whether the higher HIV-VL associated with this SNP is driving immune-dysfunction and down-stream cancer risk
- Additional analyses are required to validate the results of this study and to assess whether the association with cancer risk exists independent of HIV-infection and subsequent immune impairment

Limitations

- Overall, confidence intervals for associations with cancer were wide and, as the SNPs of interest were relatively uncommon, the number of events in persons heterozygous or homozygous for the SNP were low.
- We were also unable to validate the associations between rs6811468 and cancer in the ESPRIT cohort due to MAF of this SNP in this population.

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CONTACT INFORMATION

Daniel.dawson.murray@regionh.dk