SUPPLEMENT TO:

HLA-Cw6+ psoriasis patients show improved response to methotrexate treatment

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Methods:

Ethics statement: All data generated in this study were obtained in accordance with the Declaration of Helsinki and in compliance with local governance approval regulations (Caldicot nr C/S-AppJF1546).

Isolation of DNA. EDTA blood specimens were available through the GoSHARE programme in NHS Tayside. Of the psoriasis and PsA cohort, 123 patients were signed up to GoSHARE and of these 70 patient blood samples were available for further analysis. DNA was extracted from 1ml of blood using a QIAsymphony instrument. The concentration of DNA in the extracts ranged from 47 to 310 ng/ul, corresponding to yields of approximately 8-56 ug. In order to standardise the DNA extracts, the concentration of DNA per sample was estimated using a Nanodrop spectrophotometer. The DNA was stored at temperatures of -30^0C for long durations, and 4^0C at short term.

Preparation of Genotyping Reagent. The DNA extracts were genotyped for rs4406273 using a Life Technologies Taqman® SNP genotyping assay c/n 4351379. Samples were genotyped on a 96-well plate in a reaction volume of 5ul. Assay mix and master mix containing Taq® DNA polymerase, salts, magnesium, dNTP, and optimized reaction buffer necessary for PCR were diluted appropriately for 3ul of reagent per well. DNA (2ul of 10ng/ul) was added before the samples were centrifuged to ensure adequate mixing of the different components. The concentration of DNA and universal master mix were calculated to equal a final volume of 10ml.

PCR. The 96 well plate was inserted into the GeneAmp® PCR System 9700. 45 cycles of PCR were used for DNA amplification. An Applied Biosystems 7900HT instrument was used for allelic discrimination. In total, five samples could not be assigned to a category automatically. On inspection of these cases, two individuals

reviewed the plot and agreed on which group these samples would most likely belong to. To improve reliability of the calls, the SNP assay was repeated the following day. On viewing the allelic discrimination plot, the samples were read as having the same alleles on this repeat assay.

Hardy Weinberg check. Table S1 shows the observed and expected genotypes of the full cohort. A chi squared test was performed to rule out a difference between the observed and expected values.

Definition of Psoriasis arthritis status (PsA). Despite the widespread use of scoring systems such as CASPAR criteria, the clinical variability and number of recognized clinically distinct PsA-typical patterns means that PsA is still an essentially clinical diagnosis, made in support with x-ray diagnostics and lab studies. For the purpose of the present cohort description, we assigned a status of PsA based on the following features: first, a direct label of PsA assigned to a patient in outpatient secondary care communication directed to the GP, as documented in Dermabase. Second, verifiable clinical review by secondary care Rheumatology and inclusion of a patient in the local NHS Tayside rheumatology database (‘Rheuma’). Third, the absence of specific findings (either x-ray patterns, or CCP, or Rheumatoid factor) highly suggestive of RA being present as opposed to PsA.

Extended Results

The effect of HLA-Cw6 status on long-term MTX treatment. The retention of patients on MTX long-term beyond 1 year of treatment was analysed. The dropout rate due to inefficacy continues to decline rapidly in both genotype groups. In fact, not a single secondary failure in efficacy was observed in any of the HLA-Cw6+ patients (medium duration of observation 35 months). There is an on-going attrition rate due to limiting adverse effects, but this is not affected by HLA-Cw6 status. This was confirmed by an analysis of the spectrum of limiting AE's, shown in table S3, which did not reveal any significant differences. Thus, most of the differences in response to MTX treatment seen as a function of HLA-Cw6 status occur within the first year.

The effect of HLA-Cw6 status on long-term MTX treatment in psoriasis patients without concomitant psoriatic arthritis. The retention of patients on MTX long-term beyond 1 year of treatment for psoriasis patients without concomitant psoriatic arthritis was analysed. No patients in the HLA-Cw6 positive cohort discontinued due to a lack of primary or secondary efficacy. In both cohorts there is an on-going attrition rate due to limiting adverse effects. Similar to the whole HLA-Cw6 positive cohort it can be concluded that most of the difference in response to MTX treatment seen as a function of HLA-Cw6 status, occurs within the first year.

The spectrum of all AEs by HLA-C genotype. The spectrum of all AEs between both cohorts is shown in table S4. The table shows the spectrum of AEs in both cohorts is similar (none of the AEs occurs with statistically significant different incidence between the subgroups) with the exception of headache, which may be due to the small sample size. Notably the two common treatment limiting AEs, N/V and LFT aberration, occur in similar frequencies in both cohorts.

Supplementary Tables

Table S1. The observed and expected frequency of genotypes in the combined patient cohorts[1]

Genotype	Observed	Expected[2]
GG	43	42.4
GA	23	24.1
AA	4	3.4

[1]Data shown represents the observed frequency of each genotype in the total patient cohort.

[2]The expected genotype values shown were calculated using the Hardy Weinberg equilibrium.

Table S2. Cumulative long-term incidence treatment limiting AE spectrum in MTX treatment for parent and HLA status cohort[1]

AE term[2]	Parent cohort		HLA status cohort	
	n	Cumulative Incidence (%)	n	Cumulative Incidence (%)
LFT aberration	63	15.7	12	17.1
N/V	43	10.7	14	20.0
Fatigue	16	4.0	2	2.9
Leucopenia	13	3.2	4	5.7
Infection	9	2.2	0	0
Mouth ulcers	5	1.2	1	1.4
Dizziness	4	1.0	2	2.9
Myalgia	4	1.0	1	1.4
Headache	3	0.7	1	1.4
Alopecia	3	0.7	0	0
Rash	2	0.5	1	1.4
Diarrhoea	2	0.5	1	1.4
Dyspnoea	2	0.5	1	1.4
Gum bleeding	2	0.5	0	0
Int. Pneumonitis	2	0.5	1	1.4
Easy bruising	1	0.2	1	1.4

[1]Data shown represents all the treatment limiting adverse events reported for all treatment episodes with a cumulative incidence of ≥ 1%.

[2]Abbreviations: LFT - liver function tests; N/V - nausea and vomiting; Int. pneumonitis - interstitial pneumonitis

Table S3. Cumulative long-term incidence treatment limiting AE spectrum in MTX treatment for both patient cohorts[1]

AE term[2]	HLA-Cw6 positive		HLA-Cw6 negative	
	n	Cumulative incidence (%)	n	Cumulative incidence (%)
N/V	5	16	7	13
LFT aberration	6	19	8	14
Leucopenia	2	6	2	4
Headache	0	0	1	2
Fatigue	0	0	2	4
Diarrhoea	0	0	1	2
Rash	0	0	1	2
Mouth ulcers	0	0	1	2
Dizziness	0	0	2	4
Int. Pneumonitis	0	0	1	2
Dyspnoea	1	3	0	0
Easy bruising	0	0	1	2
Myalgia	0	0	1	2

[1]Data shown represents the total number of treatment limiting AEs in each cohort.

[2]Abbreviations: AE - adverse event; N/V - nausea and vomiting; LFT - liver function tests; Int. Pneumonitis - interstitial pneumonitis.

Table S4. Cumulative long-term incidence AE spectrum in MTX treatment for both patient cohorts[1]

AE term[3]	HLA-Cw6 positive		HLA-Cw6 negative	
	n	Cumulative incidence (%)	n	Cumulative incidence (%)
N/V	10	31	13	23
LFT aberration	6	19	13	23
Fatigue	2	6	4	7
Leucopenia	1	3	3	5
Mouth ulcers	1	3	2	4
Gum bleeding	1	3	0	0
Dyspnoea	1	3	0	0
Headache	0	0	5	9
Diarrhoea	0	0	4	7
Dizziness	0	0	2	4
Easy bruising	0	0	2	4
Vaginal discharge[2]	0	0	1	4
Rash	0	0	1	2
Int. Pneumonitis	0	0	1	2
Myalgia	0	0	1	2

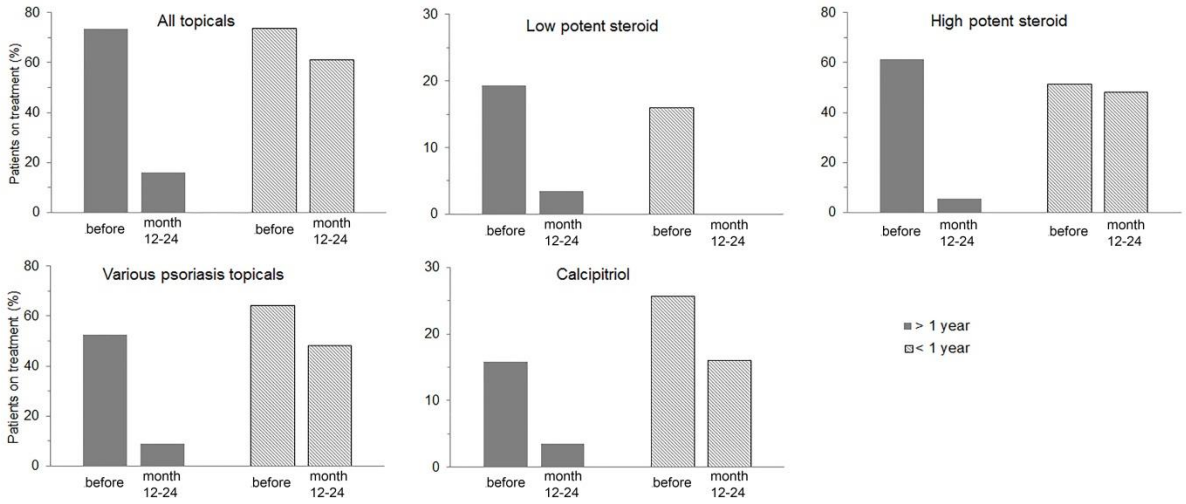
[1]Data shown represents the total number of AEs in each cohort.

[2]The percentage for vaginal discharge was calculated using the number of female treatment episodes only.

[3]Abbreviations: AE - adverse event; N/V - nausea and vomiting; LFT - liver function tests; Int. Pneumonitis - interstitial pneumonitis.

Supporting Figures

Figure S1. Consumption of topical psoriasis treatments in patients achieving 1-year of MTX duration vs. patients discontinued prior to 1 year into treatment



The percentage of patients having received a topical treatment, as indicated in the figure, in the 12-months-interval before, or the 12-months-interval starting 1 year after baseline, respectively. Dark shaded columns: patients treated > 1 year, light shaded columns: patients discontinued at < 1 year.