T regulatory cell (Treg) phenotype in Head and Neck Squamous Cell Carcinoma (HNSCC) pre- and post-treatment

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ABSTRACT
A four color flow cytometry system was employed to assess the percentage of CD4+CD25+ lymphocytes expressing FOXP3 in the peripheral circulation of 60 HNSCC patients, both pre- and post-treatment compare with 20 normal healthy controls. Additional phenotypic markers (Cytotoxic T-lymphocyte antigen; CD8 and chemokine receptor 7; CD107) were used to further characterize subsets of Tregs. We analyzed the expression of multiple subsets of T regulatory cells (Tregs) in the peripheral blood of HNSCC patients. Additionally, the effect of treatment on the expression of Tregs cell subsets was examined. In normal controls 39.6% of CD4+CD25+ cells were FOXP3+. This was significantly elevated in HNSCC patients both pre- and post-treatment (50.6% and 61.6% respectively; p<0.05). The percentage of CD4+CD25+FoxP3+ Tregs expressing CD107 was 20.6% in controls, compared to 25.4% and 34.8% in pre and post-treatment patients, respectively. The frequency of CD4+CD25+ Tregs significantly decreased to 13.9% in pre- and 10.2% in post-treatment patients compared with 18.7% in healthy controls (p<0.05). There was no association between Treg subtypes and the site of primary tumor.

This preliminary study has shown that HNSCC affects the phenotype of Treg subsets in the peripheral blood, but this is apparently not altered by treatment. Further analyses are ongoing to assess the profile of Tregs in tumor infiltrating lymphocytes and correlating these with the outcomes from the peripheral blood.

AIMS
To analyze the expression of multiple subsets of Tregs in the peripheral blood of HNSCC patients and to determine the effect of treatment on Treg subsets.

METHODS
Peripheral Blood Mononuclear Cells (PBMCs) were isolated from whole blood taken from 60 patients with resected HNSCC, both pre- and post-treatment for non-cancer related surgery. The percentage of Tregs was determined using flow cytometry following labelling of FOXP3 with fluorescent antibodies directed against the classic markers (CD4, CD25 and FOXP3). Additional markers CD107 (Cytotoxic T-lymphocyte antigen 4) and CD127 (in chemokine receptor) were used to characterize distinct subtypes of Tregs. Approval for the study was sought from South Yorkshire Local Research Ethics Committee (05/1105/35 & 06/H1105/16) and R&D approval from Hull and East Yorkshire NHS Trust.

RESULTS
In normal controls 2.4 vs 8 of the gated lymphocyte population were CD4+FOXP3+ (Fig. 1, panels A and B). This was elevated in HNSCC patients pre-treatment (3.27; 0.31%: p<0.05; Fig. 2A, Fig. 3) but decreased significantly following treatment (2.43; 0.52%; p<0.05; Fig. 2B, Fig. 3). Consequently, the level of CD4+FOXP3+ was similar to normal controls (Fig. 1B).

In normal controls 38.6 vs 16% of CD4+ Tregs were FOXP3+ and this increased in HNSCC patients, both pre- and post-treatment (50.6% and 61.6% respectively; Fig. 2A, 2B, 3A, 3B). The percentage of CD4+CD25+FOXP3+ Tregs expressing CD107 was 20.4 vs 6.1% in controls (Fig. 3) compared to 25.4 vs 25.6% in pre- and post-treatment patients, respectively (Fig. 2A, 2B, 3A, 3B). However, this difference was not significant. The percentage of CD4+CD25+FOXP3+CD107+ cells significantly decreased in both pre- (13.9 vs 1%) and post-treatment patients (25.4 vs 2%) compared with normal controls (18.7 vs 2.7%; p<0.05; Fig. 3).

There was no association between Treg subsets and the site of primary tumor data not shown).

CONCLUSIONS
HNSCC affects the phenotype of Tregs in the peripheral blood but this is apparently not altered by treatment. Further analyses are ongoing to assess the Treg profile of the full cohort of 100 patients, as well as the role of Tregs in the tumour microenvironment.

REFERENCES

Fig. 1. Dot plot of a FACS sample (R1) + lymphocytes population.