

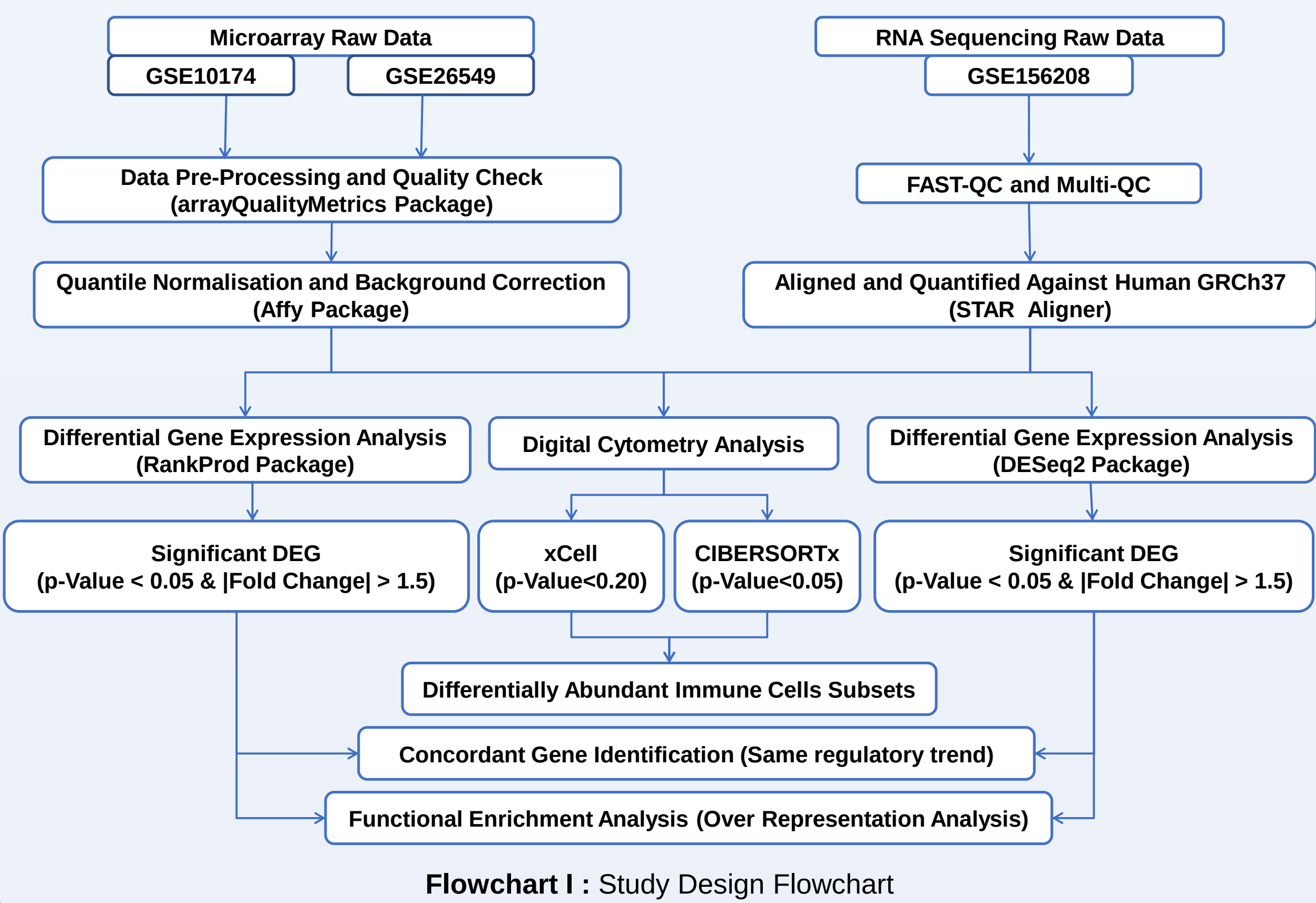
INTRODUCTION

- Oral Squamous Cell Carcinoma (OSCC) develop from precancerous lesions in a histopathological step-wise pattern from hyperplasia, dysplasia, carcinoma *in situ* to invasive OSCC.
- Progression can take up to 5-8 years and hence would require long-term follow-up for the detection/prevention of malignant transformation.
- Detection of precancerous lesions showing epithelial dysplasia are therefore key in prognostication of malignant transformation.
- Among the different treatment modalities immunotherapeutic drugs are being widely investigated for their role in chemoprevention.
- Immune profiling would provide vital information regarding the immune landscape during OSCC progression.
- To this end, we take a meta-analytical approach towards analysing publically available expression datasets of oral premalignant lesions towards identifying significant differentially regulated genes and differentially infiltrating immune cell subtypes at various pre-cancerous stages.

MATERIALS & METHODS

DATASET IDENTIFICATION

- All available archived microarray and RNA-Sequencing expression datasets were identified from ArrayExpress and Gene Expression Omnibus databases (19 unique search terms. Till 20-12-2021)
- A total of 5 datasets (microarray:3; RNA sequencing:2) datasets containing dysplastic grade annotation information were identified.
- Datasets which had profiled all three dysplastic grades, no oral cancer incidence and no history of treatment for oral cancer, were included in the study to compare the differential genes expressed across dysplastic grades within each study.
- The analysis was carried out as represented in the flowchart below.



RESULTS

Dataset	Hyperplasia	Mild dysplasia	Moderate dysplasia	Severe dysplasia
GSE10174	5	3	2	2
GSE156208	N/A	6	4	10
GSE26549	40	14	6	1

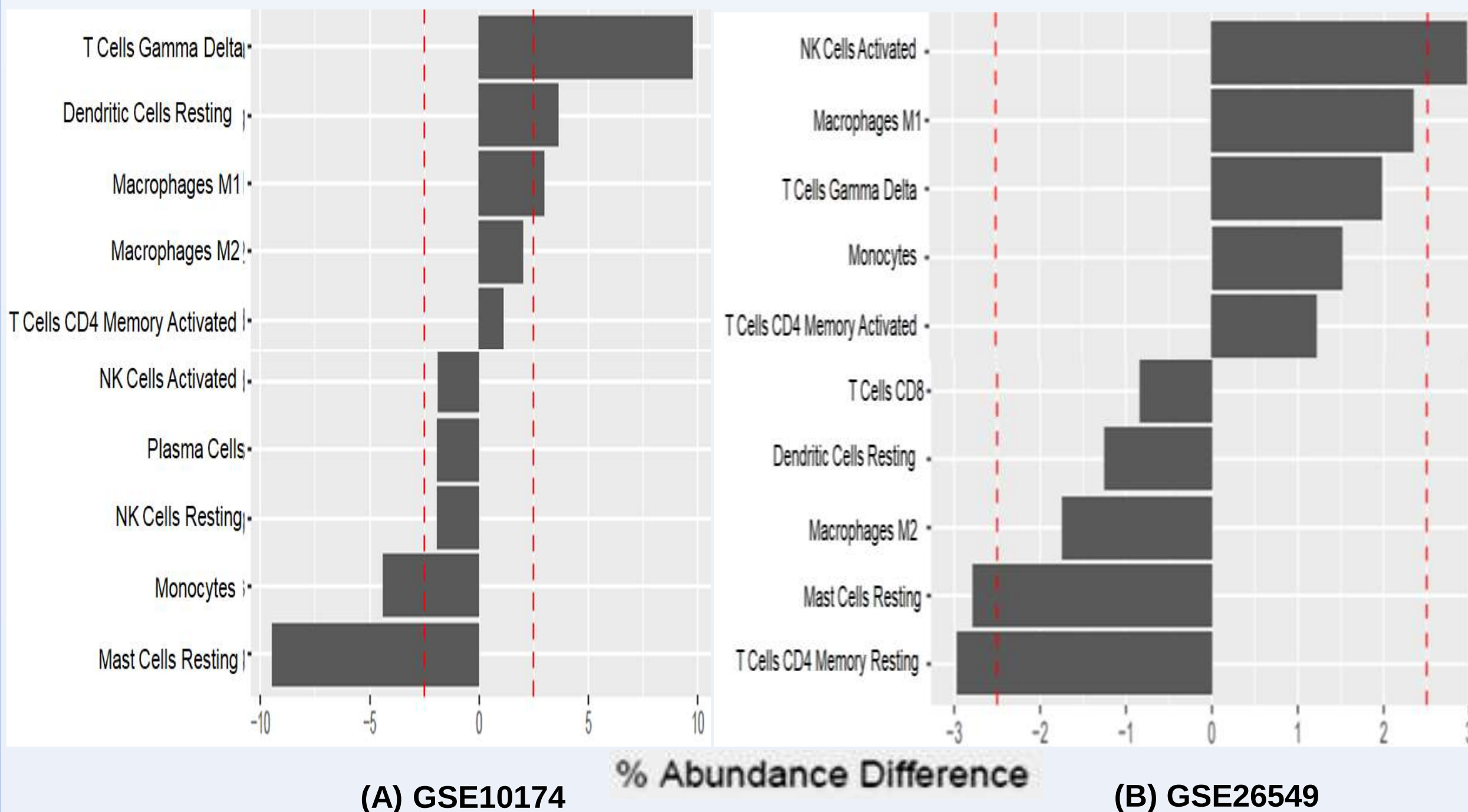
Table I : Sample type distribution across datasets

Dataset	Mild dysplasia Vs Moderate dysplasia	Mild dysplasia Vs Severe dysplasia	Moderate dysplasia Vs Severe dysplasia
GSE10174	888	1861	1733
GSE156208	501	67	325
GSE26549	254	N/A	N/A
Concordant	53	8	5

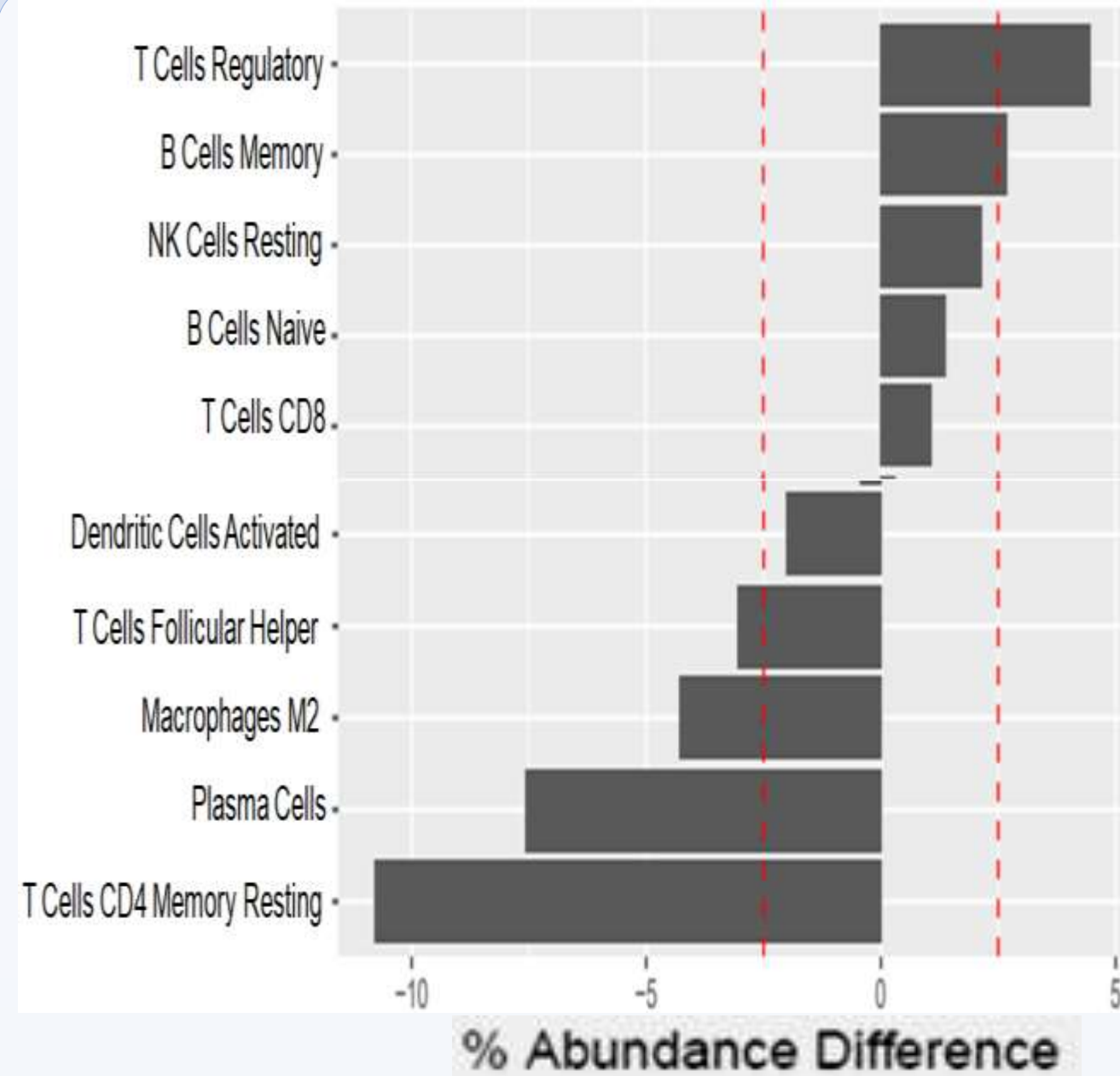
Table II : Significant genes p-Value < 0.05 and |Fold Change| > 1.5.

GSE10174		GSE26549	
Gene Set	Description	Gene Set	Description
GO:0071346	Cellular response to interferon-gamma	GO:0051607	Defense response to virus
GO:0019221	Cytokine-mediated signaling pathway	GO:0009615	Response to virus
GO:0045087	Innate Immune response	GO:0098542	Defense response to other organism
GO:0071345	Cellular response to cytokine stimulus	GO:0051707	Response to other organism
GO:0002684	Positive regulation of immune system process	GO:0043207	Response to external biotic stimulus
GO:0034097	Response to cytokine	GO:0009607	Response to biotic stimulus
GO:0006952	Defense response	GO:0031347	Regulation of defense response
GO:0002682	Regulation of immune system process	GO:0006952	Defense response
GO:0006955	Immune response	GO:0002252	Immune effector process
GO:0009605	Response to external stimulus	GO:0006955	Immune response

Table III: Top 10 GO terms from ORA using Down Regulated Genes between mild dysplasia and moderate dysplasia across two different datasets



Graph I : CIBERSORTx Top 10 differentially abundant Immune Cells between mild dysplasia and moderate dysplasia [Dotted Line : 2.5% difference in Immune abundance between sample groups]



GSE10174 dataset

- Relative abundance percentage of T-Gamma Delta Cells was 10% more in the moderate dysplastic samples than mild dysplasia
- 15% difference observed in the severe dysplastic samples when compared to moderate dysplasia.

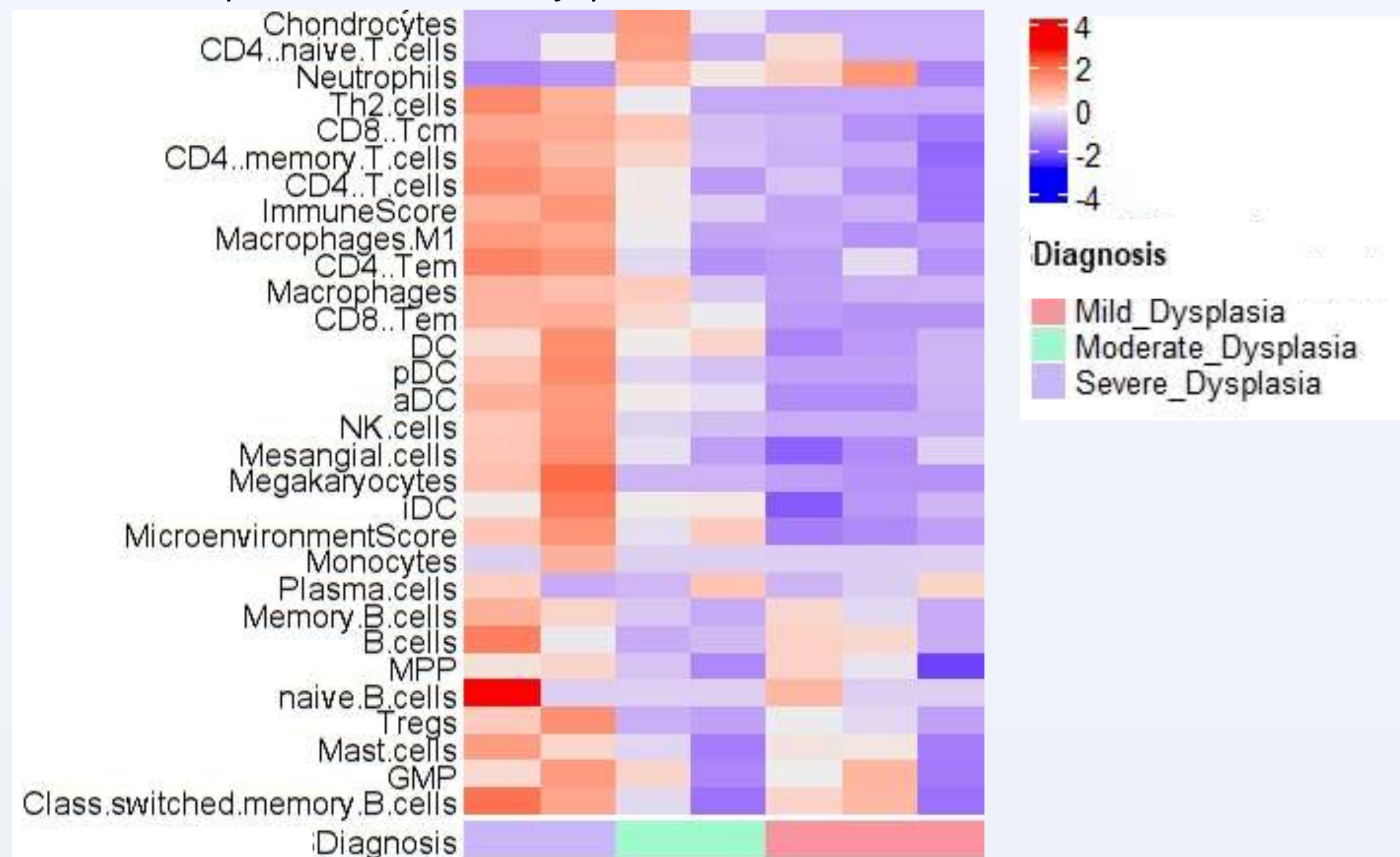
GSE26549 dataset

- Similar trend observed between the dysplasia grades of mild dysplasia when compared to moderate dysplasia albeit to a lesser percentage difference of 2%.

Graph II : CIBERSORTx Top 10 differential abundant Immune cells between mild dysplasia and moderate dysplasia in GSE156208

GSE156208 dataset

- T Regulatory cells show a greater infiltration in the moderate dysplastic samples (5%) compared to mild dysplasia
- Severe dysplastic samples show a 4% difference in infiltration with T Cell CD4+, when compared to mild dysplastic and a 15% increased abundance when compared to moderate dysplasia.



Heat map I : Differentially abundant immune cells by xCell for GSE10174

- Similar results were obtained from the xCell immune abundance results as is evident from the representative heatmap from one of the studies GSE10174 shown above.
- The results of the concordant differentially regulated genes, the functional over Representation analysis of the identified significantly dysregulated genes and the digital cytometry results obtained using two different methods do **show the preliminary evidence of the change in immune abundances within the dysplastic samples being related to T-Cells.**

CONCLUSIONS

- Previous studies have reported the presence of an increase in the abundance of Immune cells within the Oral Pre-Malignant Disorder groups¹.
- Others have reported distinct subtypes within the OPMD's as immunological and classical, based on the immune signaling pathways and gene expression profiles².
- However, these studies involved a large number of Hyperplastic tissues within the cohorts and usually treated the different grades of dysplasia as a singular cohort.
- Recent, systematic reviews on immune checkpoint inhibitors show a lack of evidence among OPMD's and the lack of studies where the immune checkpoint molecule expression was compared to OSCC's/normal controls³.
- However, not much is known regarding the differences in the immune abundances within the three dysplastic grades of OPMD's apart from Immuno-Histochemical Studies performed specifically targeting subsets of specific immune cells.
- In light of this background, the results of the meta analytical approach taken in this preliminary study, indicate the presence of immune related biological processes playing a significant role in dysplasia progression.
- The results of the digital cytometry methods show the relative abundance differences of different immune cells within a sample to be different as dysplasia progression is observed; the most notable of these immune cells would be T Cell whose relative abundances showing significant variations between the dysplastic grades.
- The identification of 66 significant dysregulated genes with fold change above 1.5 are indicative of consistently differentially expressed genes within the disease group under study.

FUTURE WORK

- The markers identified need to be validated for their clinical relevance; studies are currently ongoing

REFERENCES

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