

## Abstract:

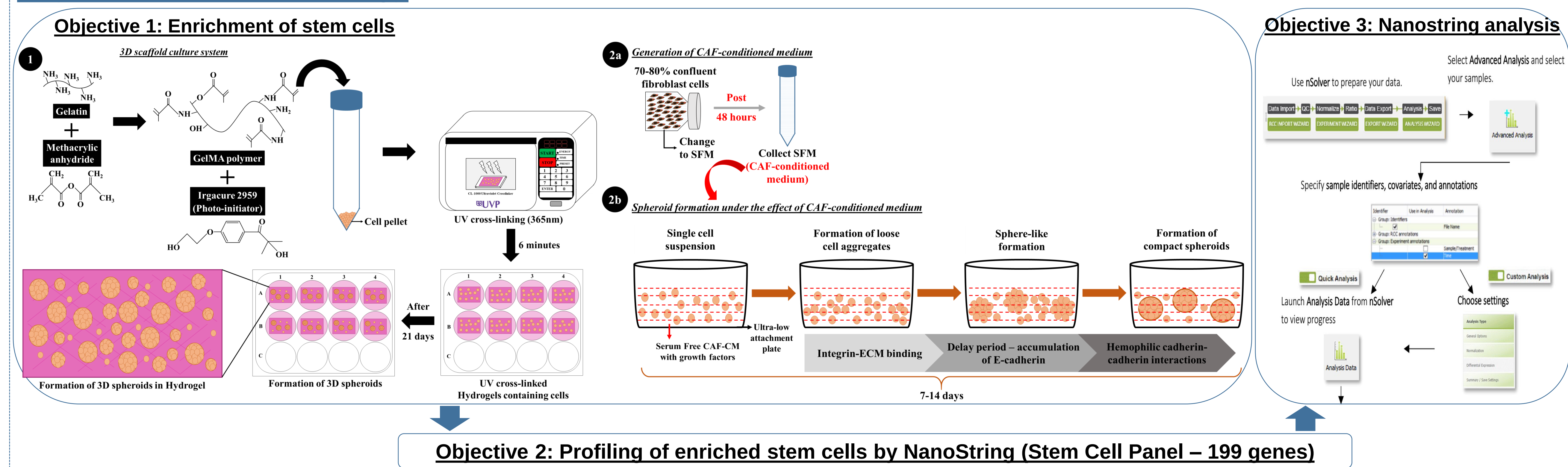
**Introduction:** Understanding the cellular and molecular basis of carcinogenesis will be a primary step towards devising effective chemo preventive strategies. This proposal aimed to characterize CSCs during oral cancer progression and elucidate the effect of fibroblast interactions. The primary outcome of the project will be biomarkers that can be clinically applied in prediction and early detection and/or will provide possible candidates as novel chemoprevention targets.

**Methodology:** The human cell lines HaCaT (non-neoplastic) and DOK (dysplastic) were used in the study. Assessment of self-renewal capacity was carried out in 3D cultures and under the effect of CAF-conditioned medium (CAF-CM) by spheroid formation. The spheroids generated were used for NanoString Analysis with Stem Cell Panel (199 genes) (outsourced to TheraCUES Innovations Pvt. Ltd., Bangalore). The analysis of the NanoString data was carried out using R programming. Correlation plots were used to compare the different batches of the samples. The analysis of NanoString data was carried out using nSolver 4.0 software. The *p*-value adjustment for the experiment was carried out using the Benjamin-Hochberg method. The genes identified will be validated in respective cell lines and patient samples.

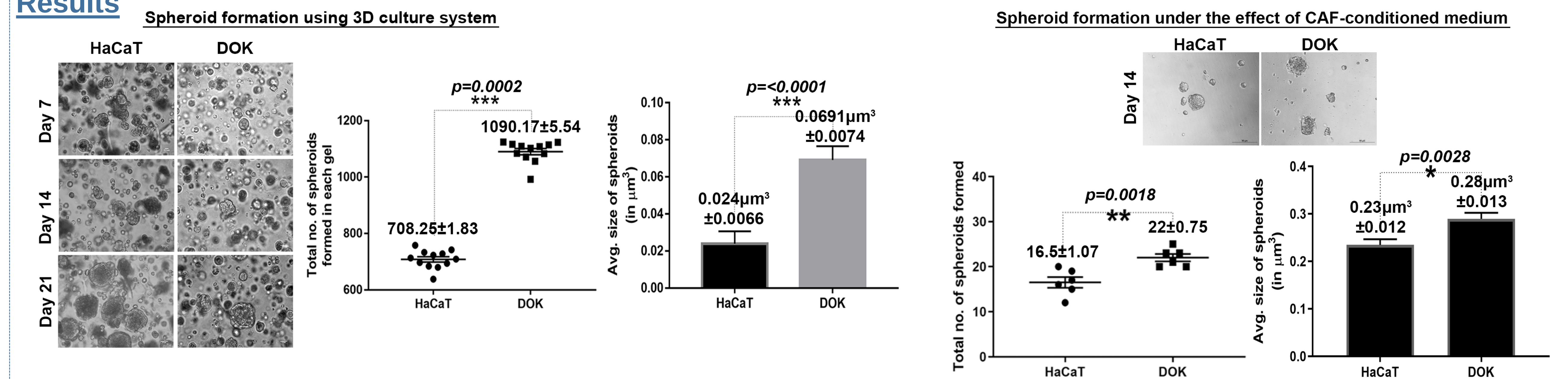
## Introduction

- Two-step process of oral carcinogenesis well established (*Reibel, 2003*); precursor lesions: leukoplakia, erythroplakia (*Axell et al., 1996; Jemal A, 2010*).
- Tumor initiating cells (TICs)/cancer stem cells (CSCs) implicated in oral carcinogenesis (*Subin et al., 2017*) and field cancerization (*Simple et al., 2015*) – characterizing CSC-niche interactions – newer insights into carcinogenesis.
- Hence, molecular and functional assessment of SCs from different stages of oral carcinogenesis will provide a deeper insight into the possible distinctive features of SCs along with the feasibility of their use as molecular predictors/novel targets.**

## Objectives and Methodology



## Results

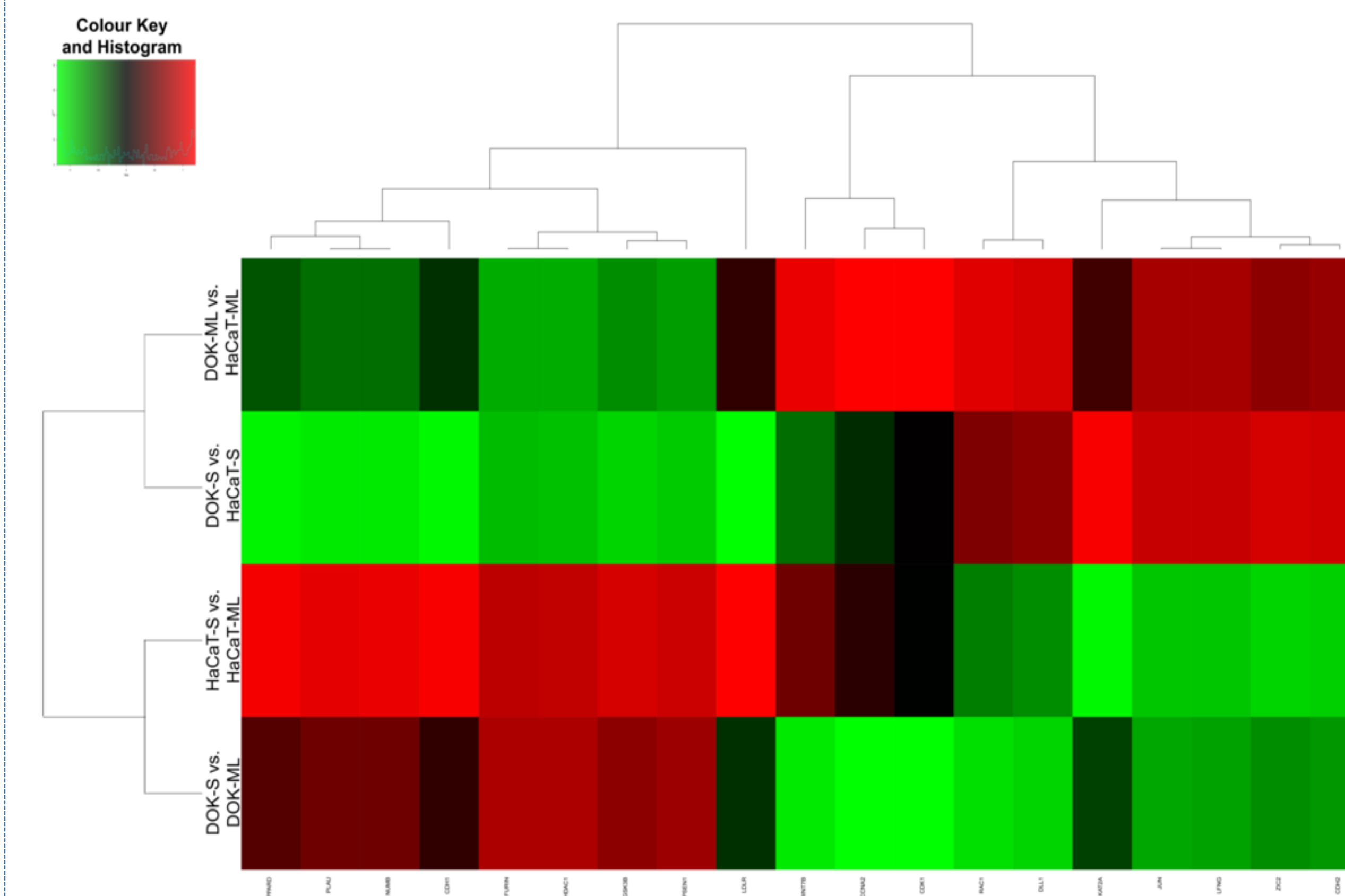


## Enhanced sphere formation in HaCaT and DOK cells using 3D culture system and under the effect of CAF-conditioned medium

## Enhanced stem cell markers in 3D spheroids

- Spheroids vs. monolayer cells**
  - HaCaT: *CCND3* down-regulated; DOK: *CCND3*, *WNT7A* down-regulated
- DOK vs. HaCaT spheroids:**
  - Total: 13 genes (up-regulated: 5, down-regulated: 8)
  - WNT7A* down-regulated in DOK spheroids when compared to DOK monolayer and HaCaT spheroids
- DOK vs. HaCaT monolayer cells:**
  - Total: 13 genes (up-regulated: 5, down-regulated: 8)
  - Unique: *FZD2* up-regulated; *FZD3*, *CSNK1G1*, *PPP2R5C*, *DVL3*, *NUMB* down-regulated

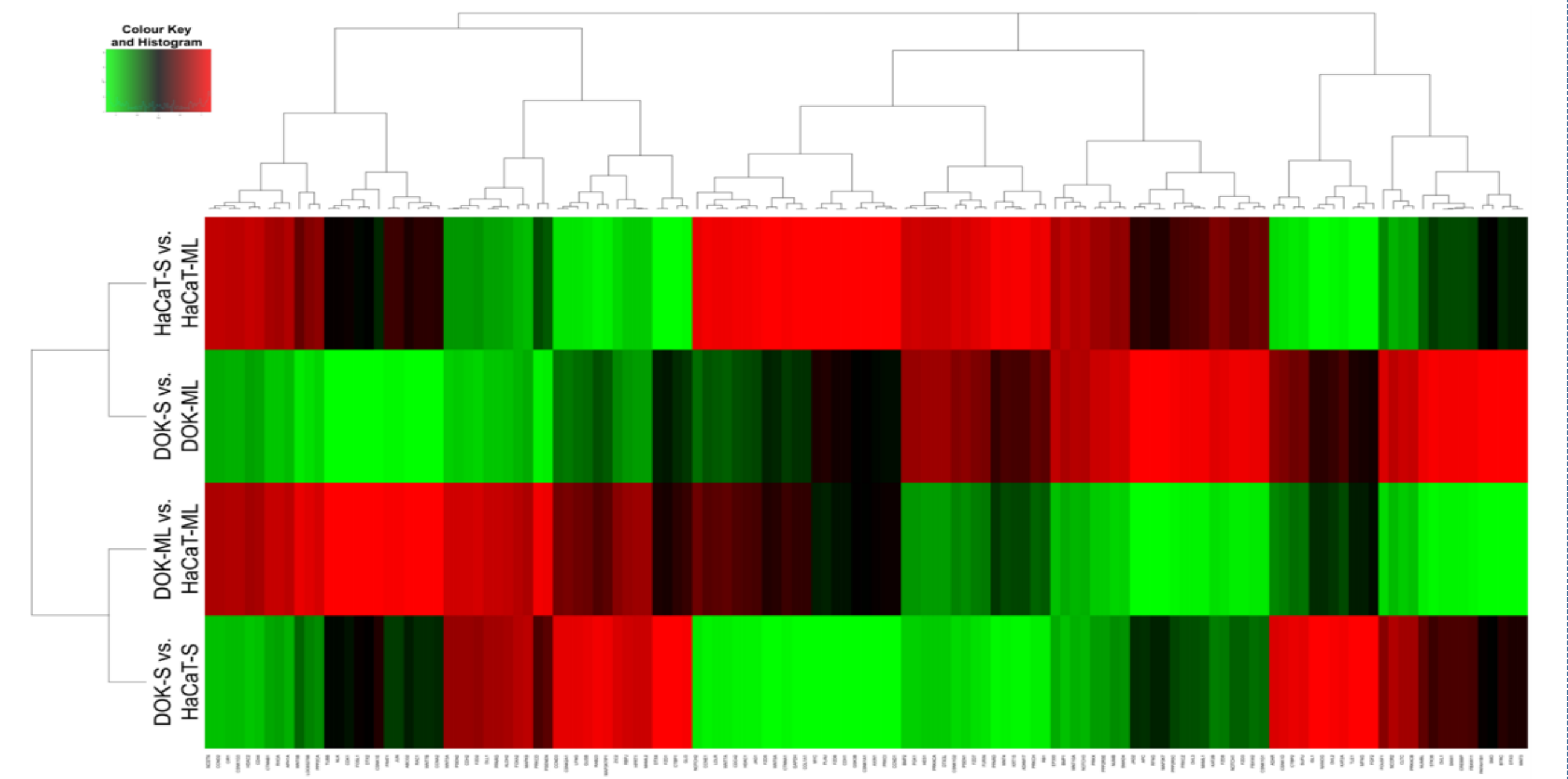
## Heat map representing different comparisons in CAF-induced spheroids



## Conclusions and Future Directions

- Non-neoplastic, dysplastic cells form spheres in 3D scaffold culture system** and under the effect of CAF-conditioned medium.
- 3D and CAF-induced dysplastic spheroids were significantly high in number (*p*<0.005) and larger in size (*p*<0.05) as compared to non-neoplastic spheroids.
- A 15-marker panel differentiated between HaCaT 3D spheroids and DOK 3D spheroids.
- Marker panel identified that are specific to non-neoplastic/dysplastic CAF-induced spheroids.
- Validation of markers in respective cell lines and annotated patient samples currently ongoing.

## Heat map representing different comparisons in 3D spheroids



## Enhanced stem cell markers in CAF-induced spheroids

- Spheroids vs. monolayer cells:**
  - CDC42, ALDH2: uniquely up-regulated in HaCaT and DOK spheroids respectively
  - Cyclin D2 and Cyclin D3: down-regulated in DOK spheroids
  - CD44, PLAU, KRT15, Wnt and Notch pathway markers: commonly up-regulated in both spheroids
- DOK vs. HaCaT spheroids:**
  - Total: 56 genes (up-regulated: 28, down-regulated: 28)
  - Collagenase and CTBP1: uniquely up-regulated
  - ALDH2, BMP2, FZD5, WNT5A: up-regulated in DOK spheroids compared to monolayer and HaCaT spheroids
  - ABCG2, CSK1G3, LDLR, PRKC1: down-regulated in DOK spheroids when compared to monolayer cells and HaCaT spheroids
- DOK vs. HaCaT monolayer cells:**
  - Up-regulated: PSEN2, WNT5B; down-regulated: CSNKIG1, NOTCH