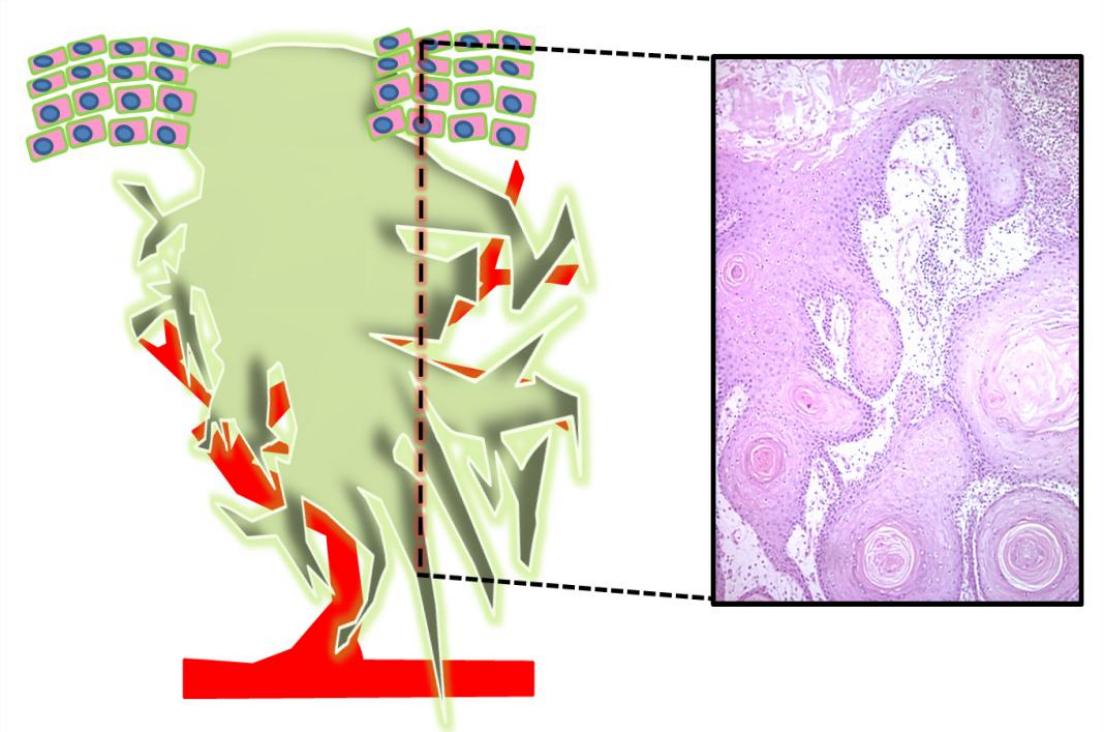
“Tumor Initiating Cells in Human Cutaneous Squamous Cell Carcinoma”

Jonathan Vogel, M.D.
Dermatology Branch



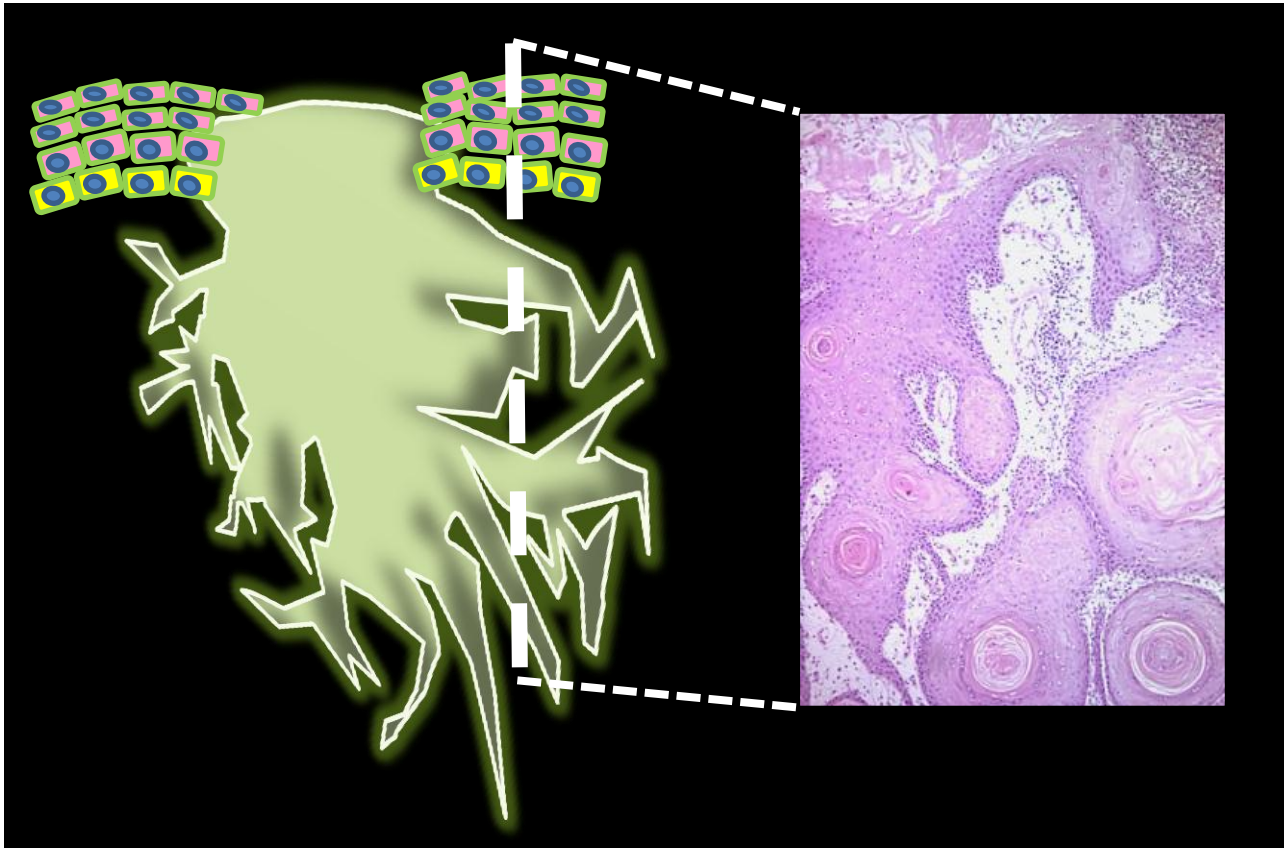
Introduction

Human cutaneous squamous cell carcinomas (SCC) exhibit a heterogeneous morphology with a developmental hierarchy of proliferating and differentiating cells may be maintained by a distinct population of cancer stem cells or tumor initiating cells (TIC).

In vitro tissue culture assays and in vivo animal models that can accurately recapitulate the human cancer were developed to identify and characterize TIC .

Demonstrate that a small subset of human SCC cells (~1%) expressing a prominin-1 (CD133) epitope are highly enriched for TIC in human SCC

Human Squamous Cell Carcinoma (SCC)



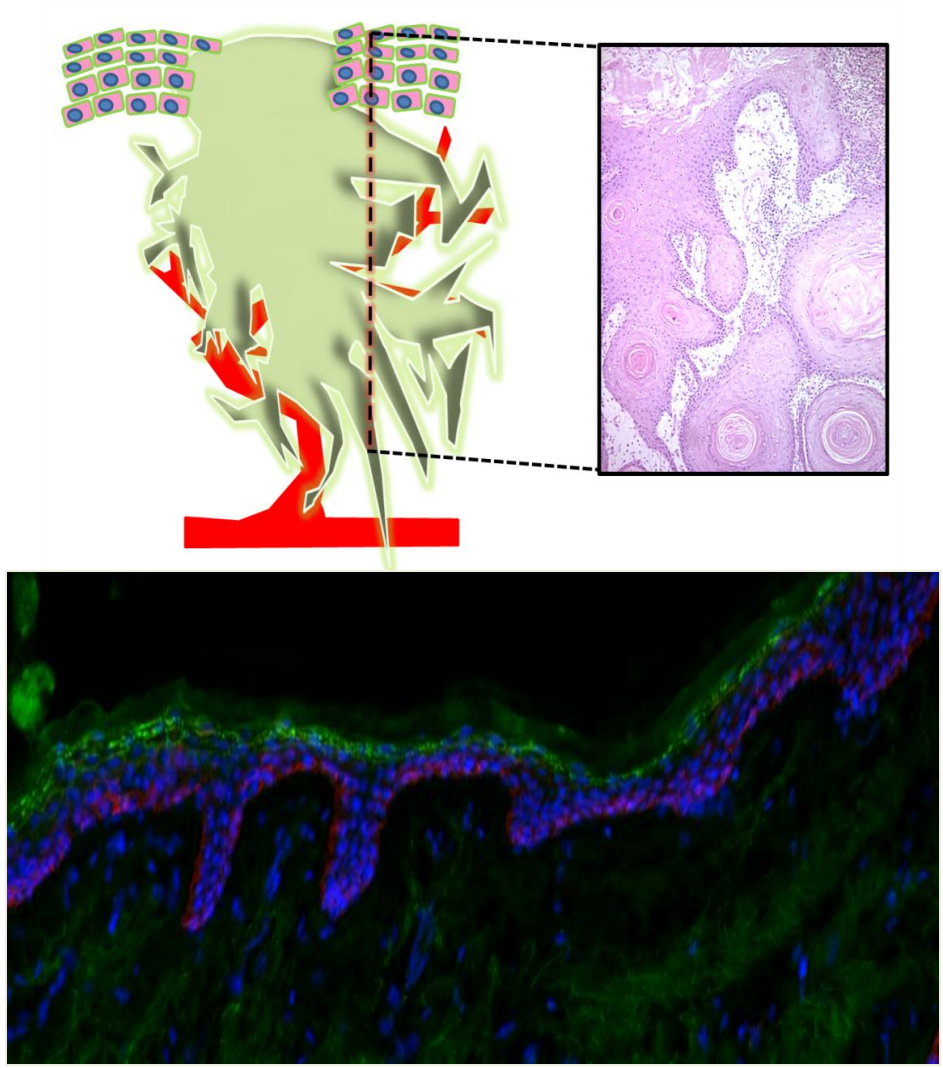
Squamous cell carcinomas and basal cell carcinomas represent more than 10^6 cases per year, about 25% SCC

Etiology due to DNA damage secondary to sun and environmental exposure

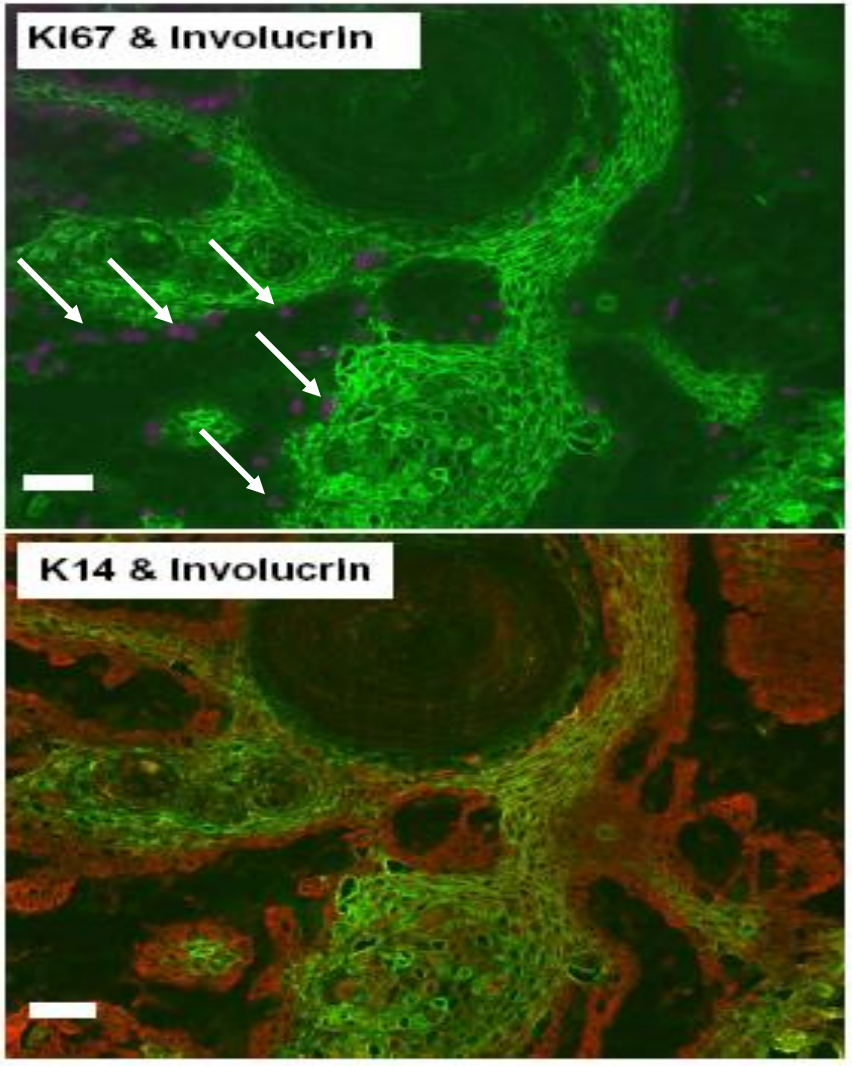
High incidence of SCC metastasis in transplanted and immunocompromised patients

Proliferating dysplastic keratinocytes invade locally as a mass with finger-like tumor projections invading into tissues

SCC continue to differentiate with Ki67+ proliferating cells located at the periphery of SCC tumor projections



Normal Skin: K5 & Involucrin



Squamous cell carcinoma
Ki67+, K14 and Involucrin

Isolation and characterization of tumor initiating cells in SCC

Human SCC

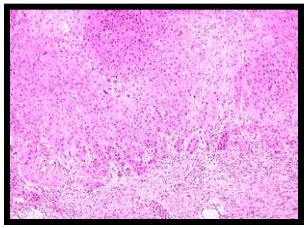
I Isolation of single cell suspension

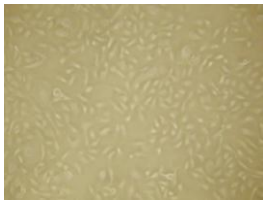
II Separation of cells based on cell surface markers

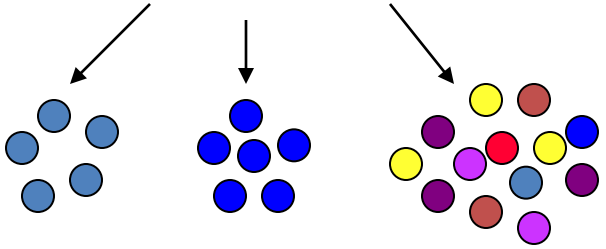
III Assess for growth/tumor formation

In vitro assay

In vivo tumor initiation assay

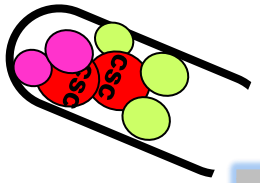


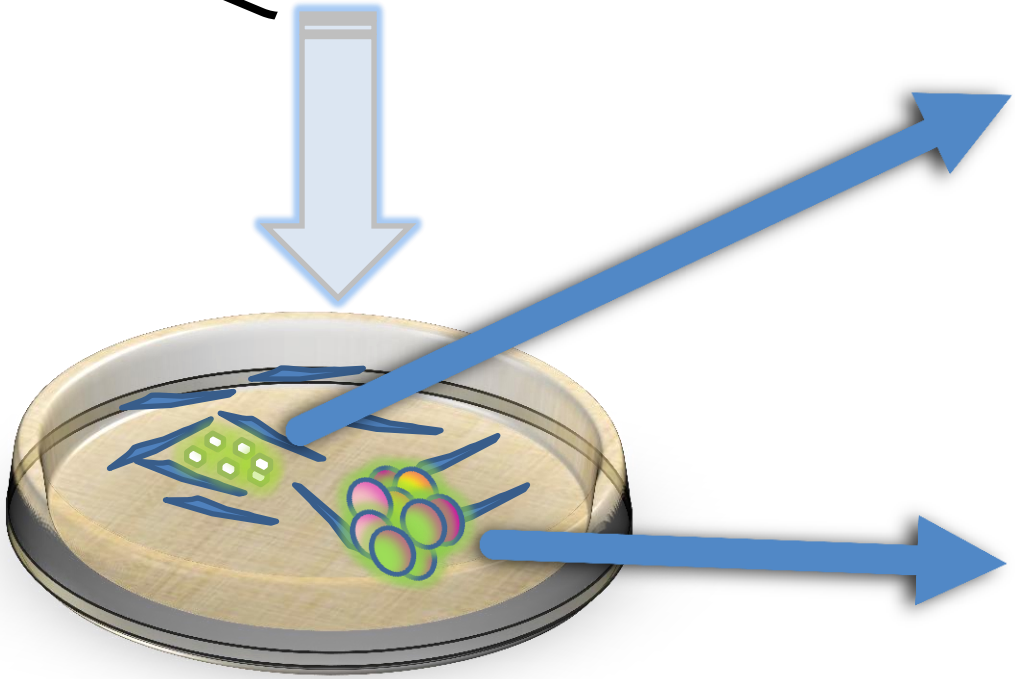




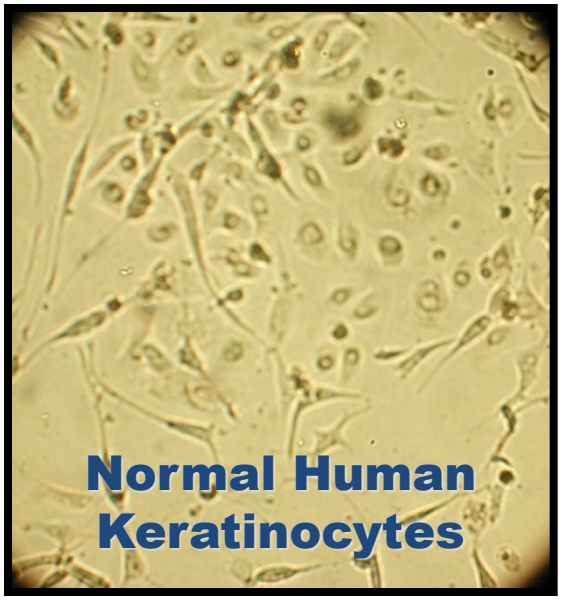
Human SCC form spheroid tumor cell colonies in culture

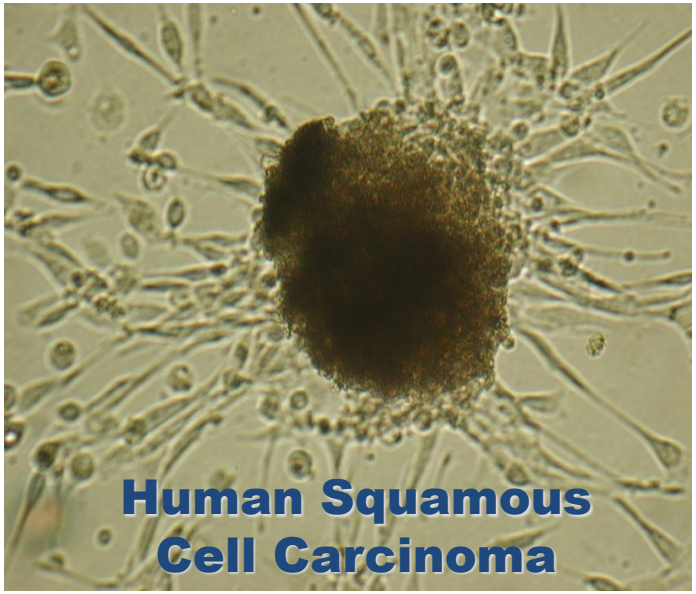
Bulk Human Squamous Cell Carcinoma Cell Suspension





Tissue Culture Plate with Irradiated 3T3 feeder layer





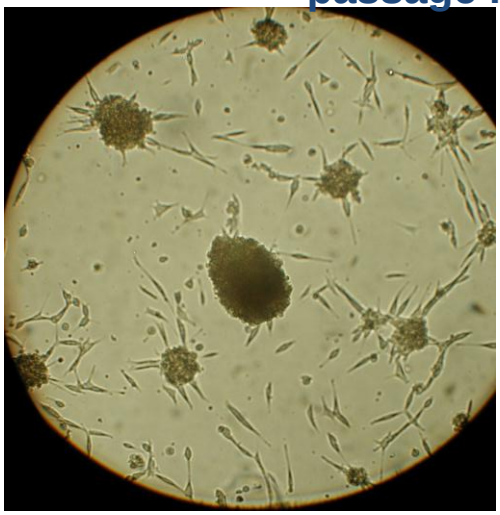
SCC spheroid colonies could be serially passaged without increase or decrease in colony numbers noted

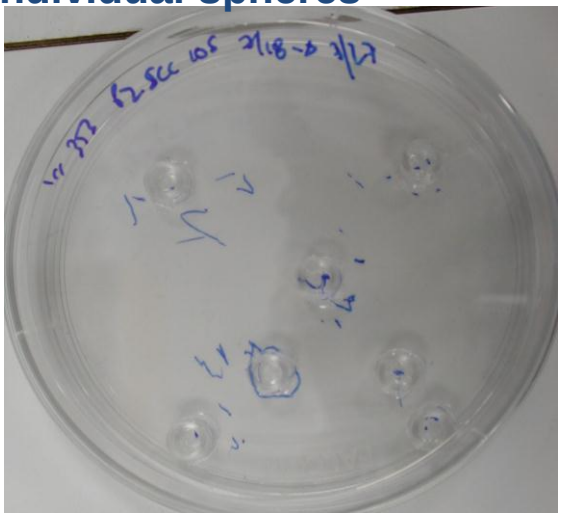
Whole plate trypsinized and passaged

SCC

Input	10^5
# 1° colonies	49
# 2° colonies	48

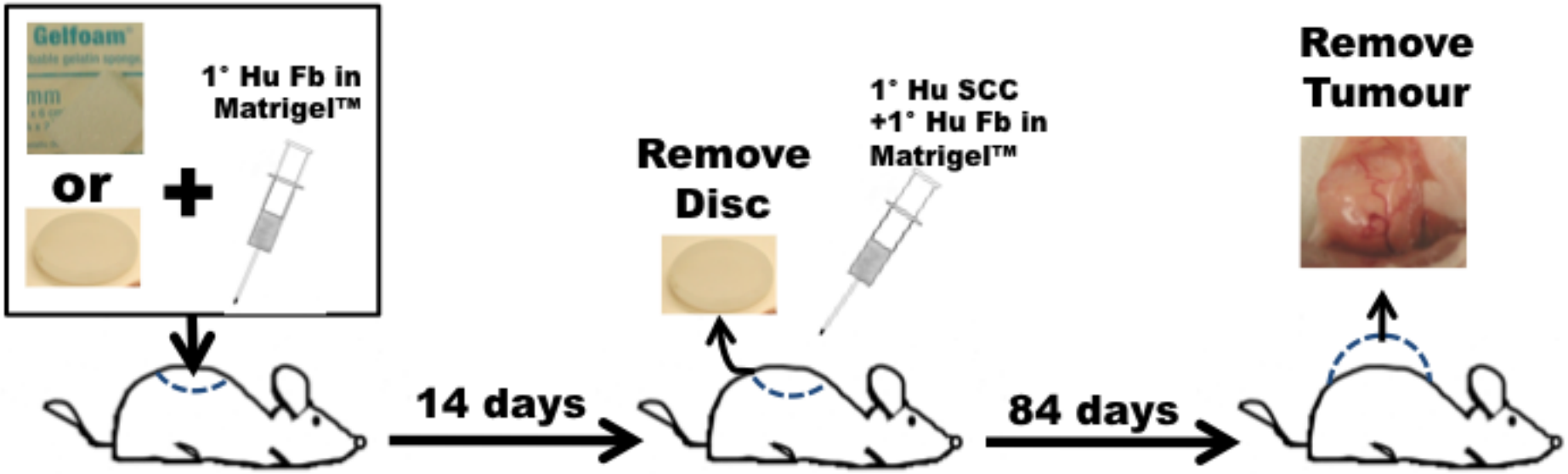
Cloning cylinders used to trypsinize and passage individual spheres





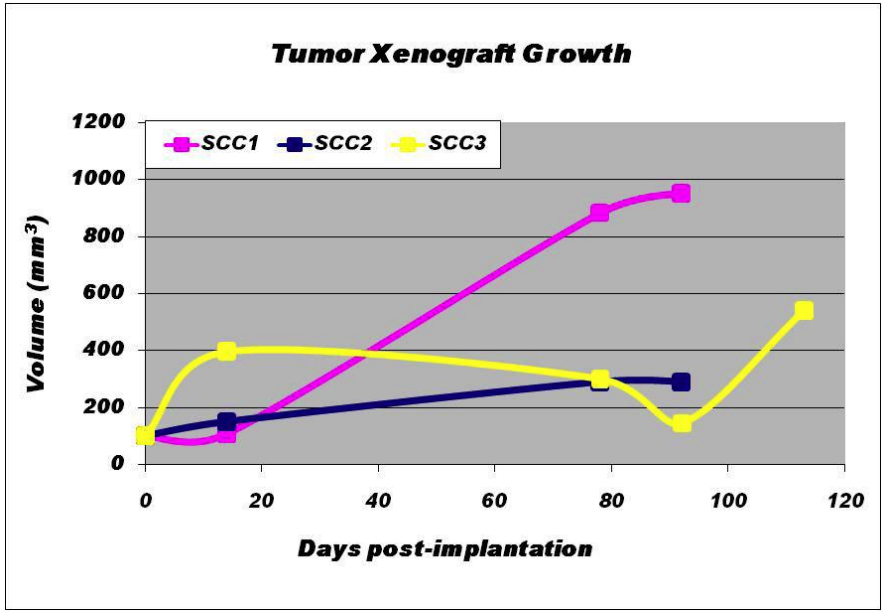
In Vivo Model for Human SCC Initiation

Successful xenografts of human SCC cell suspensions required extensive "humanization" of the graft site

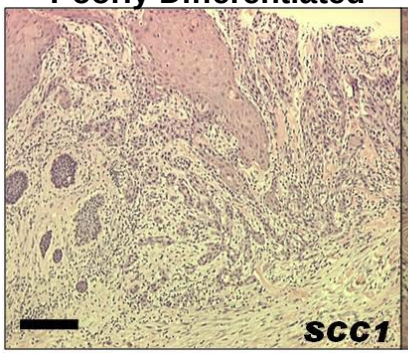
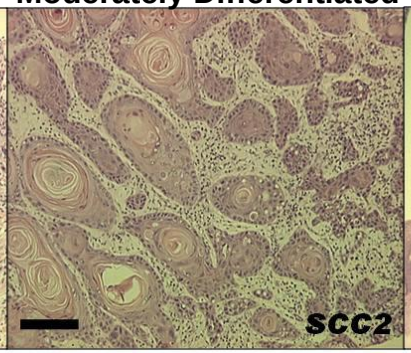
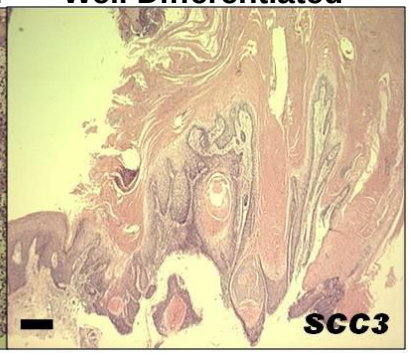
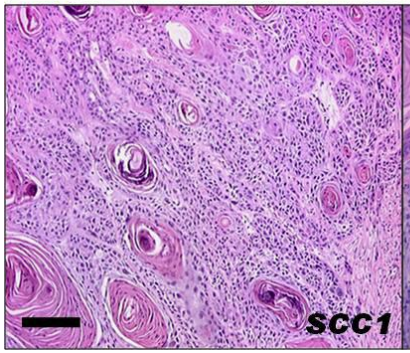
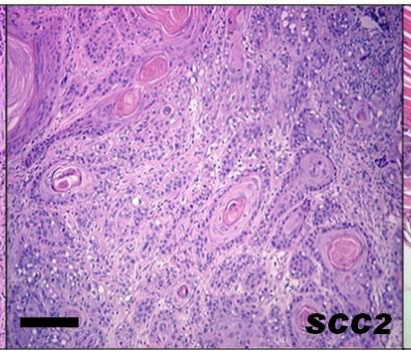


Establishing this in vivo assay required 140 separate human SCC samples and 155 individual mouse xenografts over a 3 year period

Xenograft SCC tumor growth rate and histology was similar to original SCC tumor



SCC1 – Poorly Differentiated
SCC2 – Moderately Differentiated
SCC3 – Well Differentiated

	Poorly Differentiated	Moderately Differentiated	Well Differentiated
Xenograft Tumor			
Original Tumor			

Isolation and characterization of tumor initiating cells in SCC

Human SCC

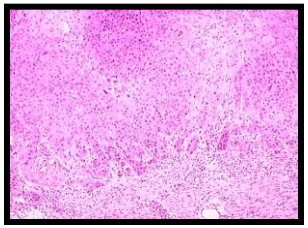
I Isolation of single cell suspension

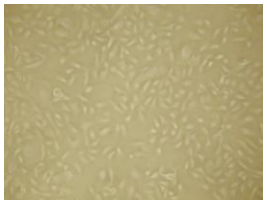
II Separation of cells based on cell surface markers

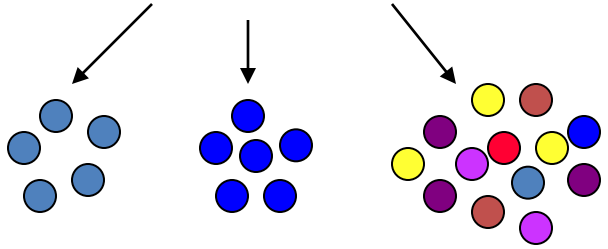
III Assess for growth/tumor formation

In vitro assay

In vivo tumor initiation assay



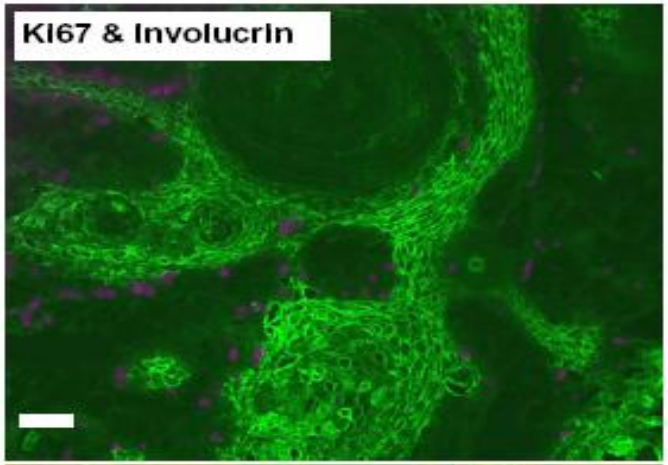


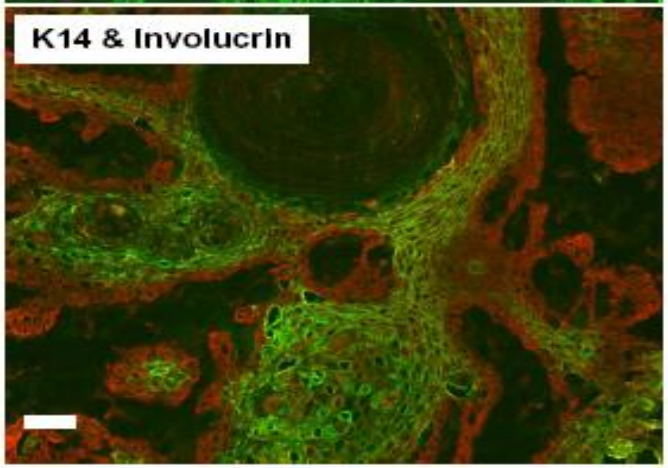


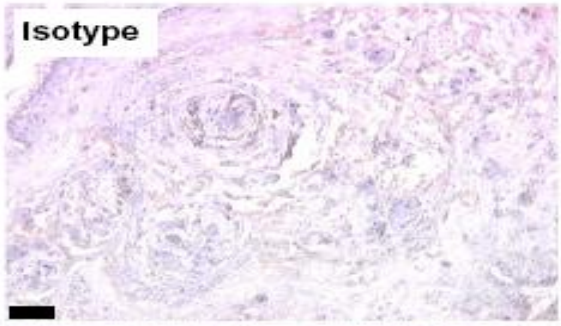
Potential Cell Surface Markers for Tumor Initiating Cells

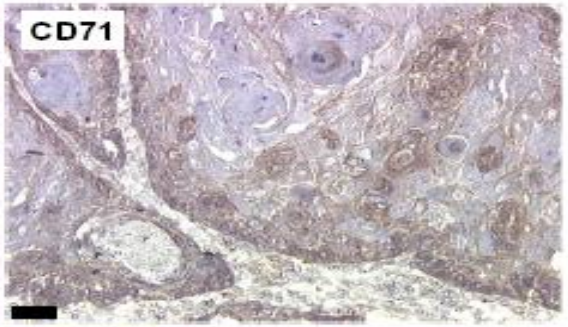
1. Previously, panels of cell surface markers were identified for human keratinocyte stem cells in hair follicles (**$CD200^{hi}24^{lo}34^{lo}71^{lo}146^{lo}$**)

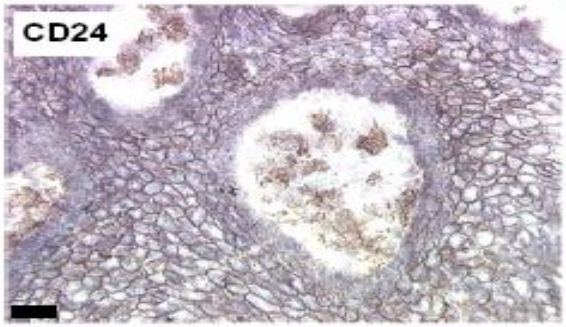
2. The **CD44** and CD24 cell surface markers have been used to isolate tumor initiating cells from fresh human cancer specimens, including breast, head and neck, and pancreas. The glycosylated **CD133** cell surface marker has also identified tumor initiating cells in primary human cancer, including brain (medulloblastomas and glioblastomas), colon, and pancreas.

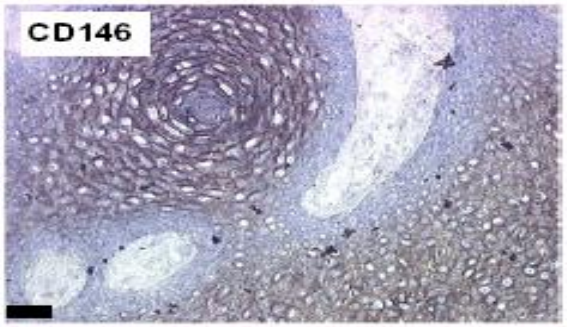


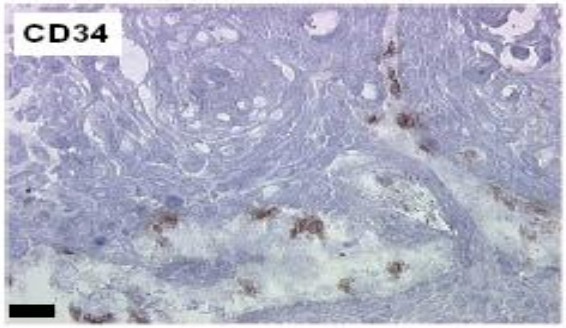


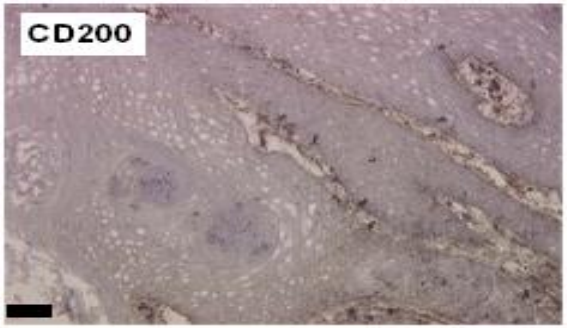




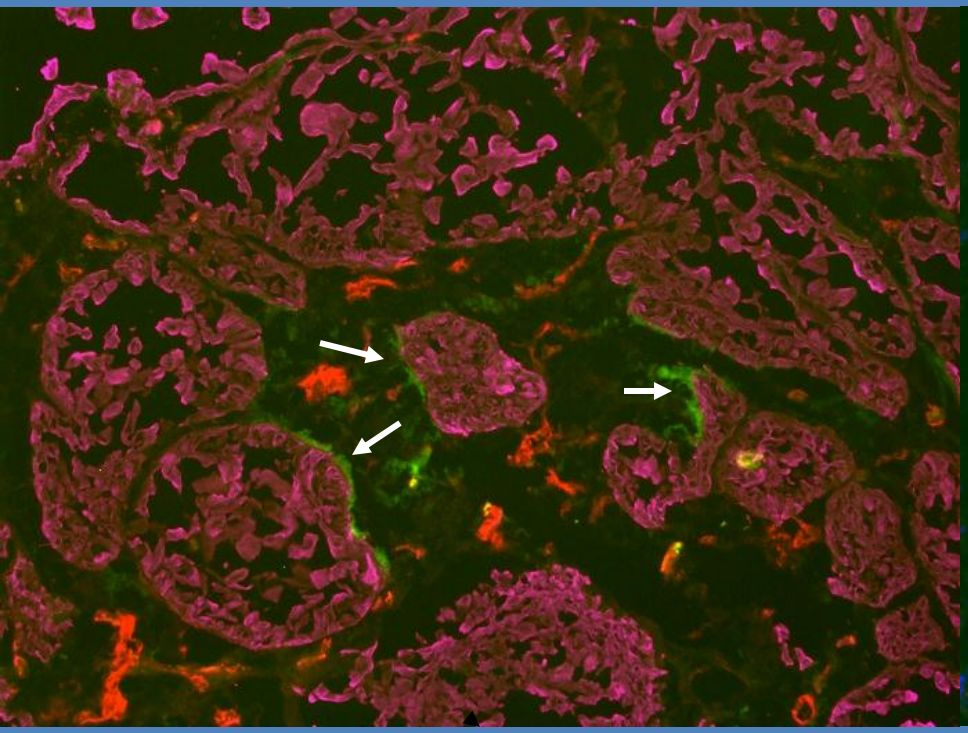




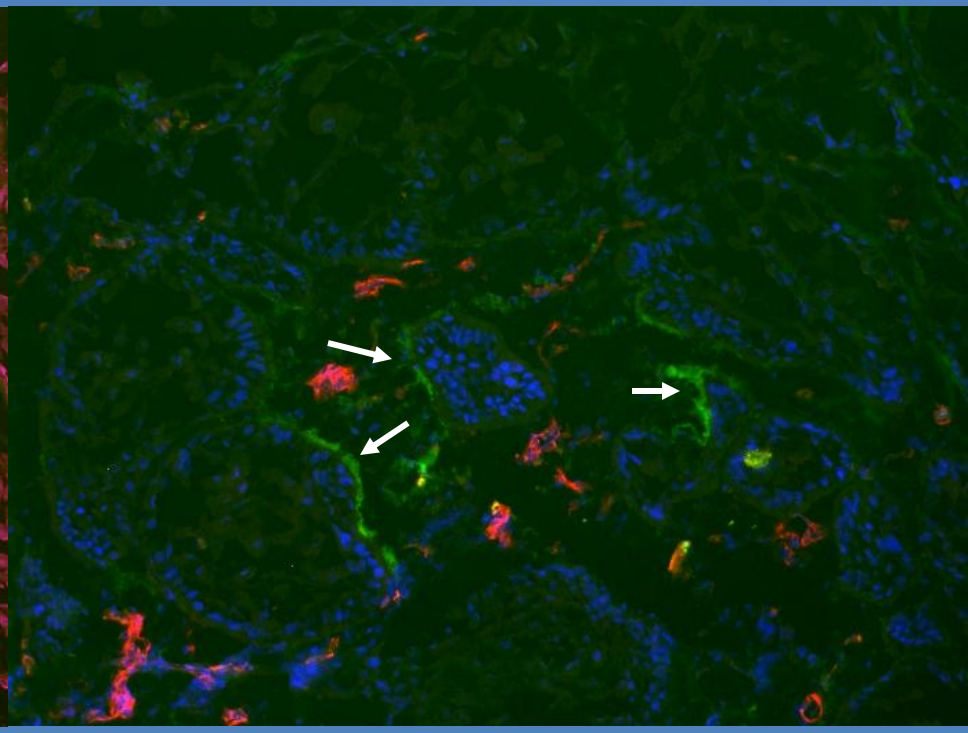




CD133 was expressed on scattered cell clusters in the proliferating layer of the human SCC tumor projections



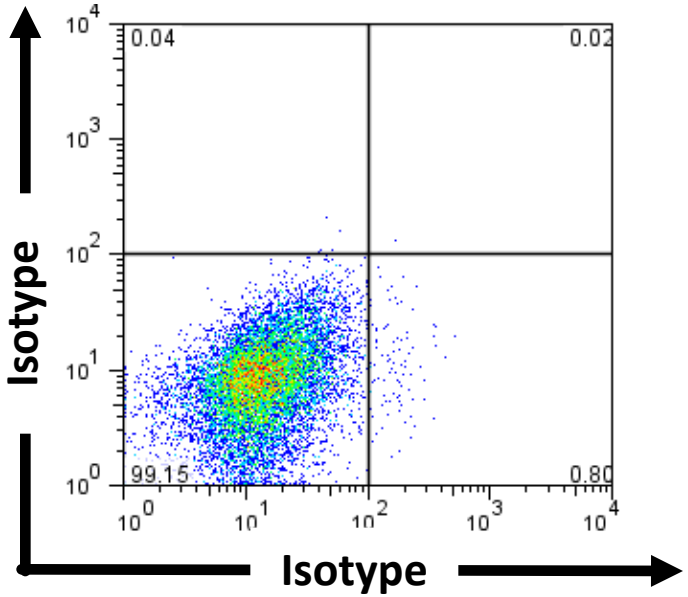
CD31 (pecam)
CD133 (promin1)
Keratin

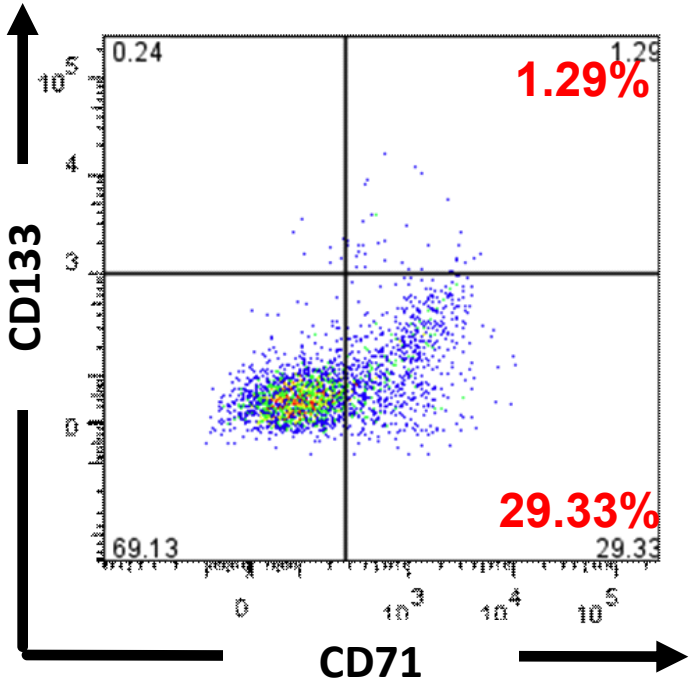


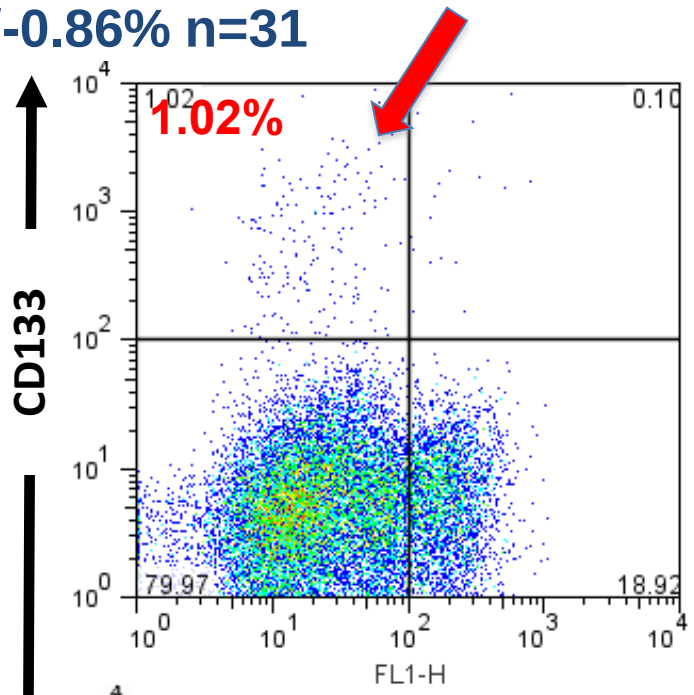
CD31 (pecam)
CD133 (promin1)
Dapi

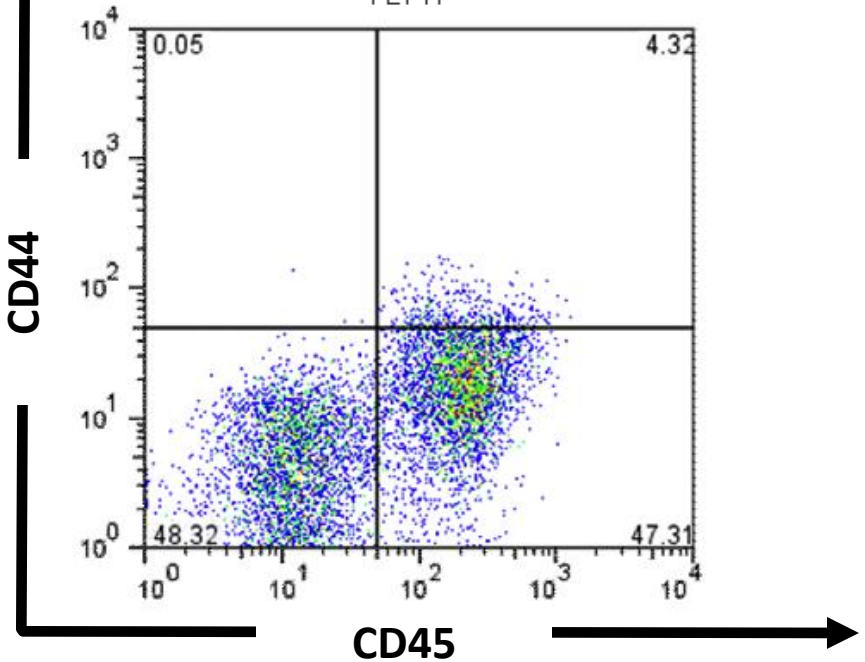
CD133+ cells represent a rare subset of human SCC cells

Mean 0.81% +/-0.86% n=31

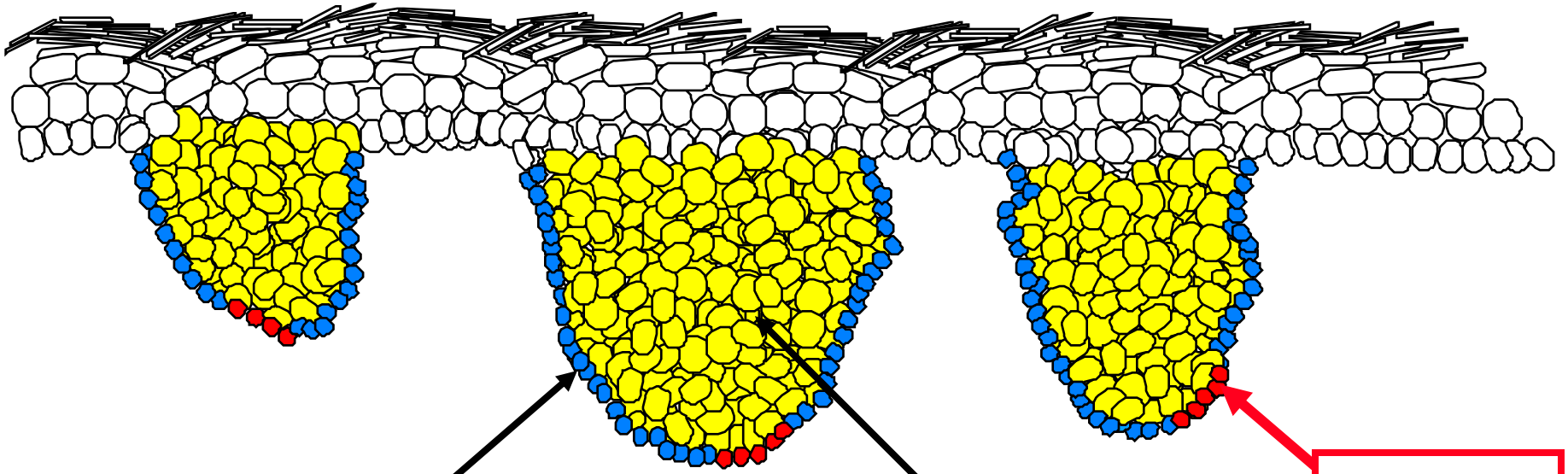








Summary of Cell Surface Markers in SCC



CD133-Hi

Proliferating SCC periphery
CD71-hi
(CD24- and CD146-)

Differentiating inner SCC
CD24-hi and CD146-hi
(CD71-)

CD200+ cells are not present in SCC and CD44+ cells were CD45+

Isolation and characterization of tumor initiating cells in SCC

Human SCC

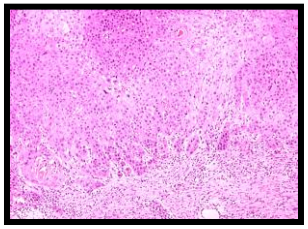
I Isolation of single cell suspension

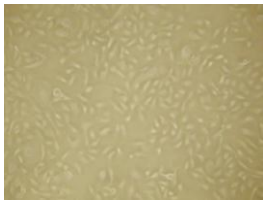
II Separation of cells based on certain characteristics

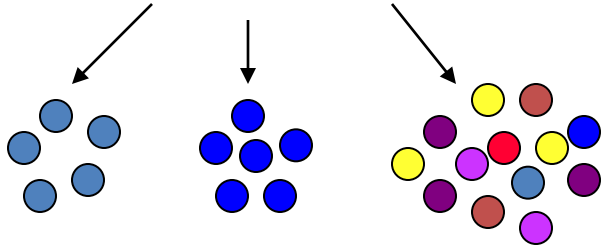
III Assess for growth/tumor formation

In vitro assay

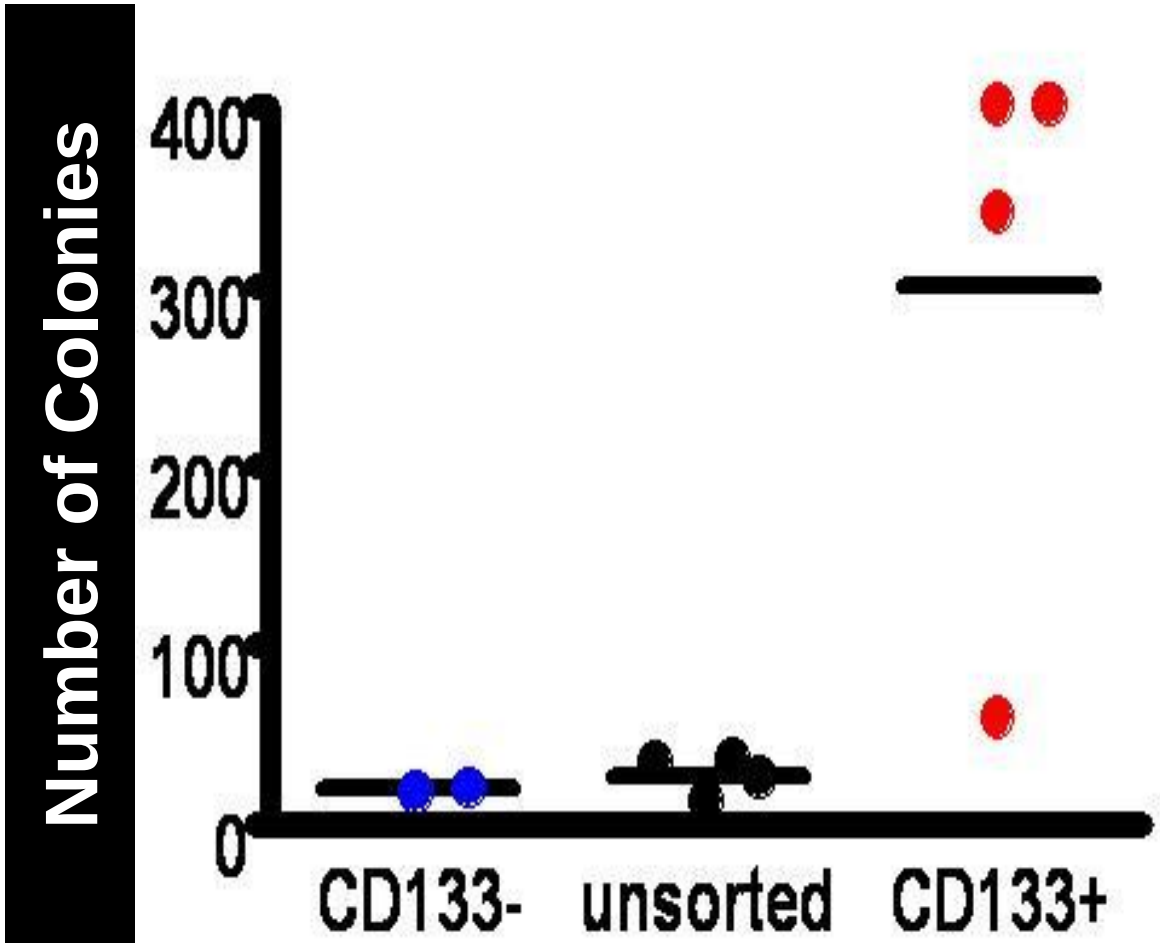
In vivo tumor initiation assay

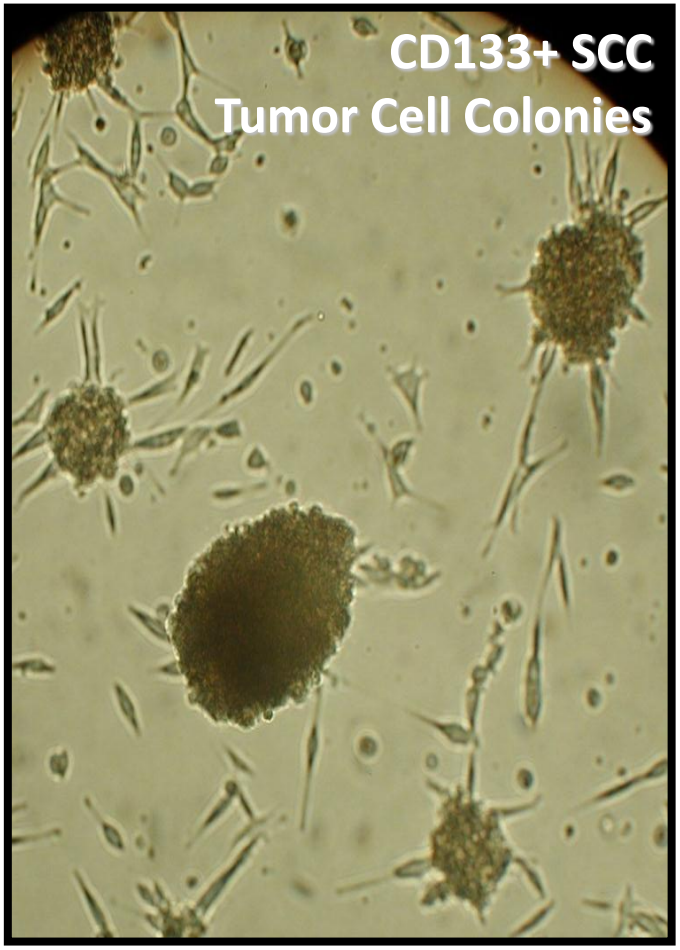






CD133+ cells isolated from SCC are enriched for spheroid colony formation





Isolation and characterization of tumor initiating cells in SCC

Human SCC

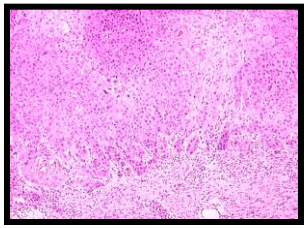
I Isolation of single cell suspension

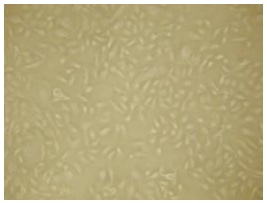
II Separation of cells based on certain characteristics

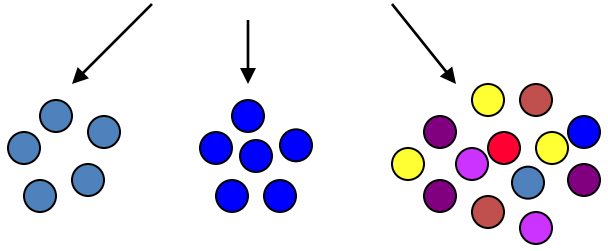
III Assess for growth/tumor formation

In vitro assay

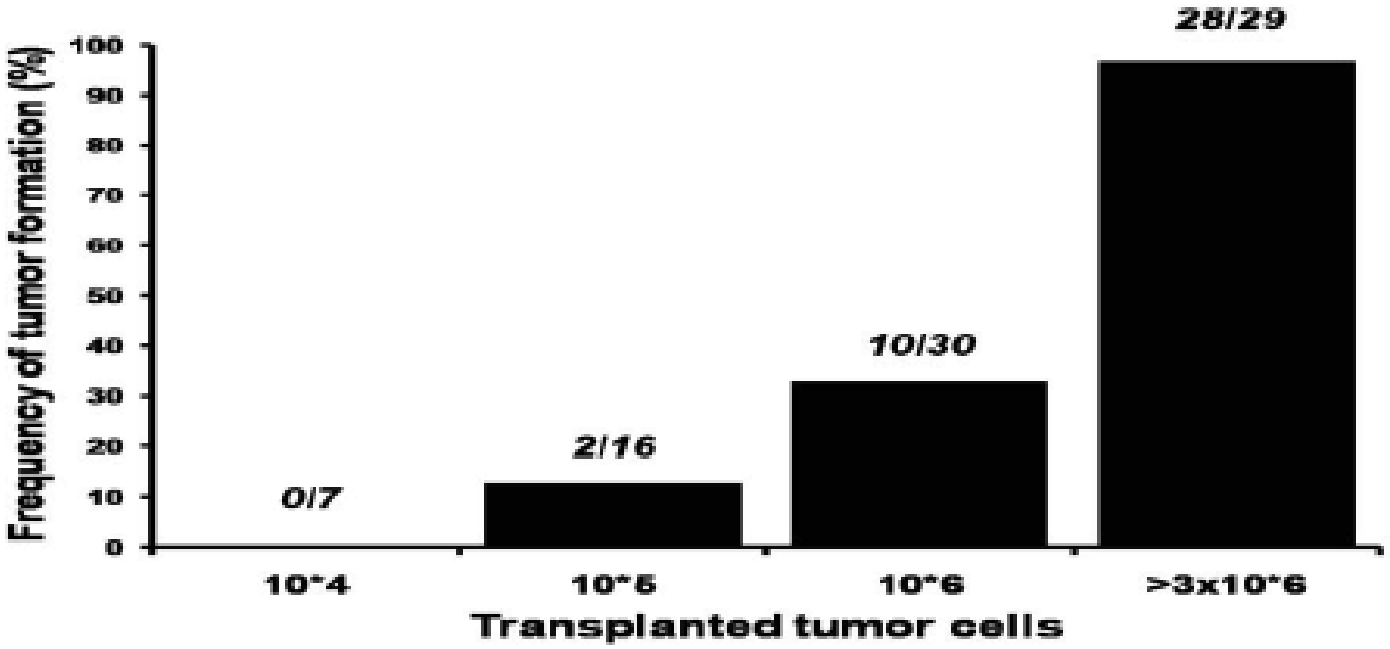
In vivo tumor initiation assay







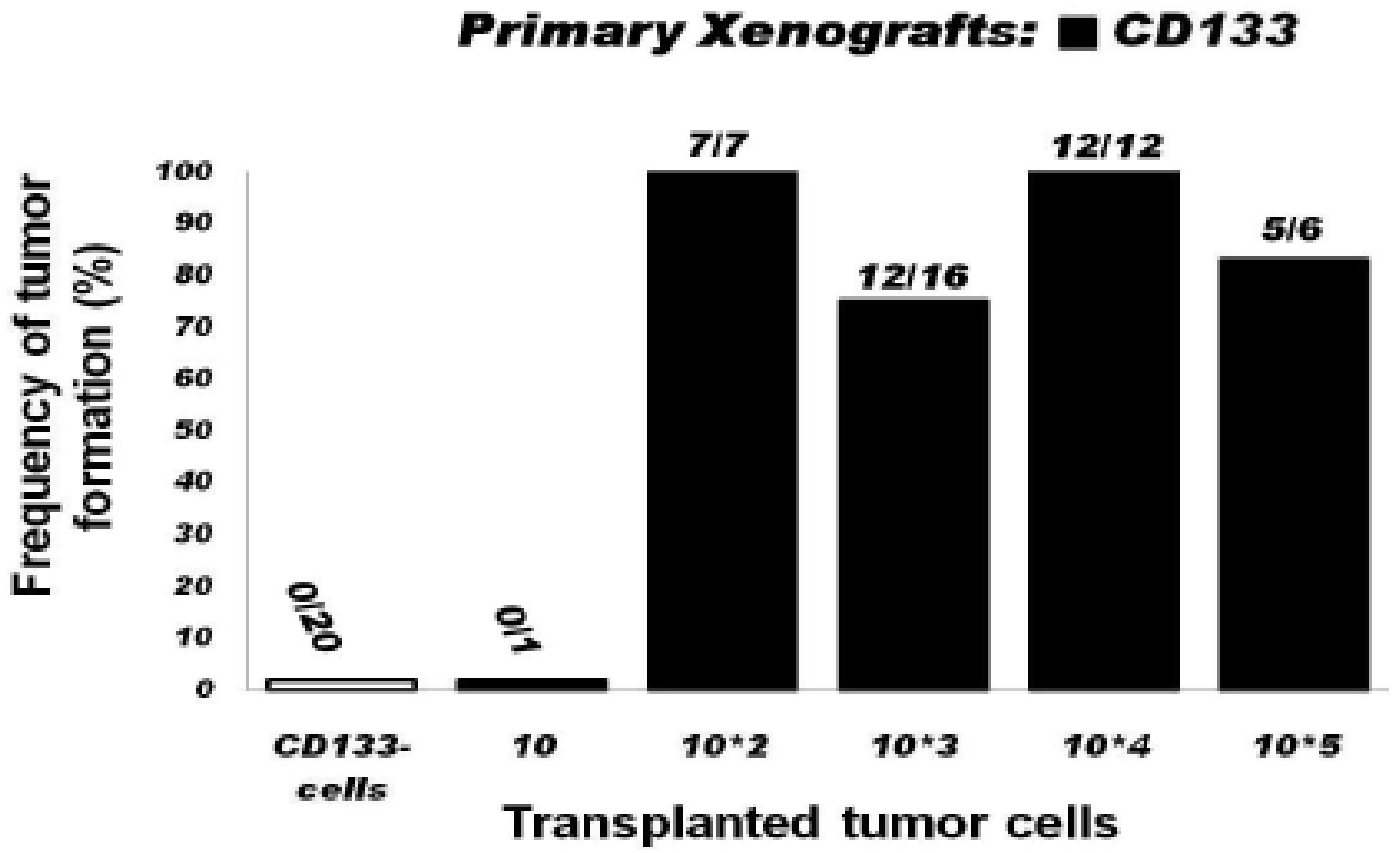
Tumor growth was dependent on the number of unsorted human SCC cells xenotransplanted



82 Total Xenographs into Nude Mice

TIC frequency = 1 / 1,400,000 Total SCC cells

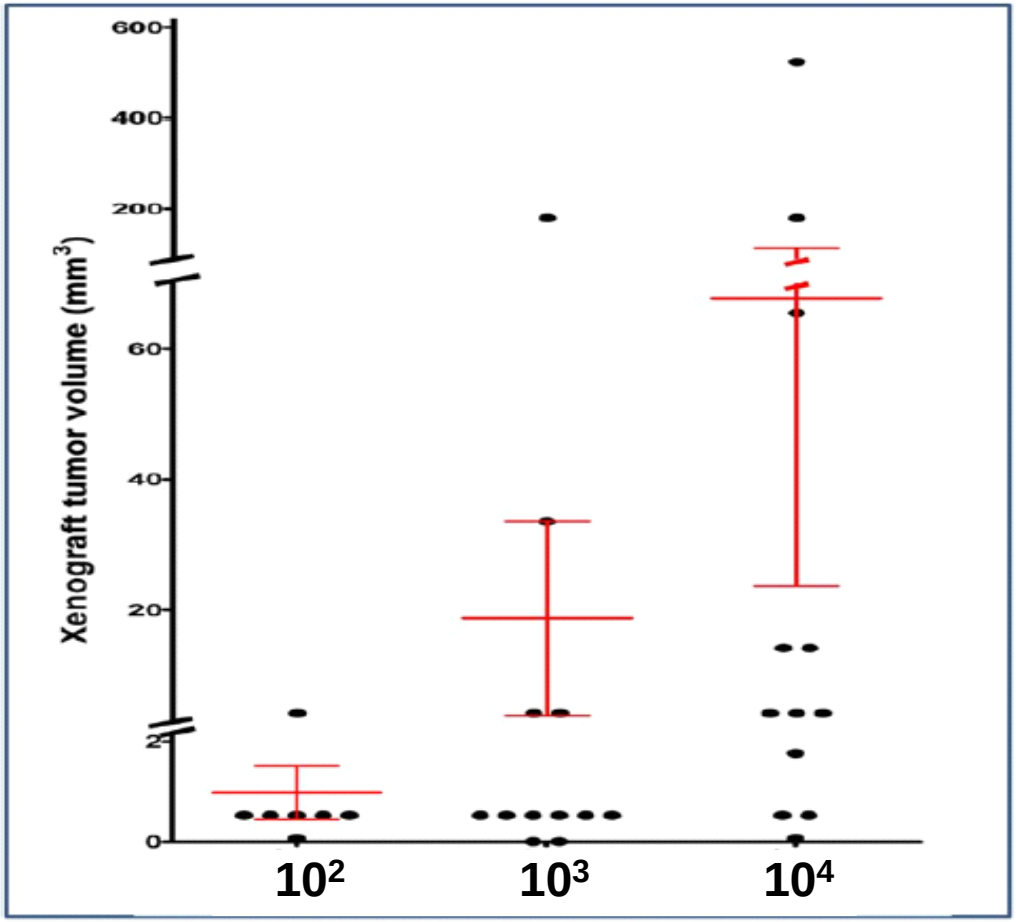
Xenotransplanted CD133+ SCC cells are highly enriched for TIC



42 total xenografts from 28 different human SCC specimens

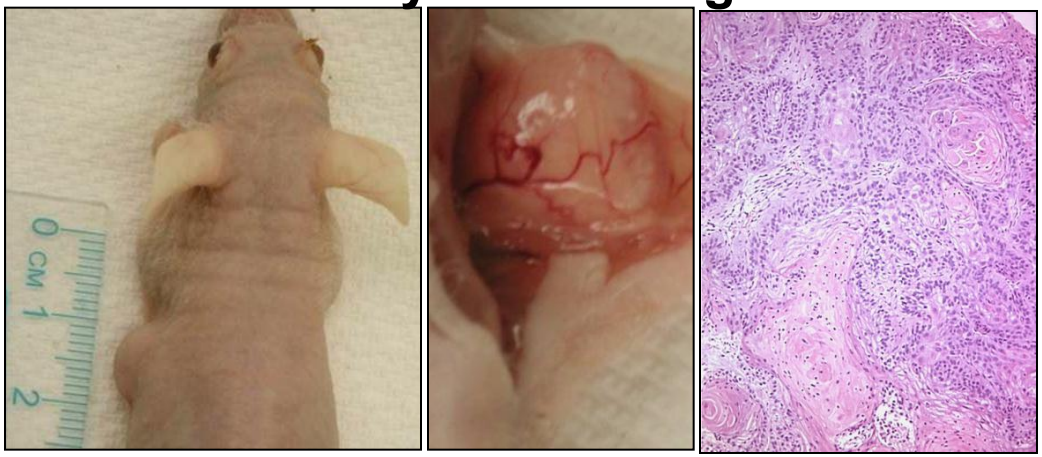
TIC frequency = 1 / 483 CD133+ cells

The number of implanted CD133+ SCC cells can determine xenograft size (at 12 weeks)



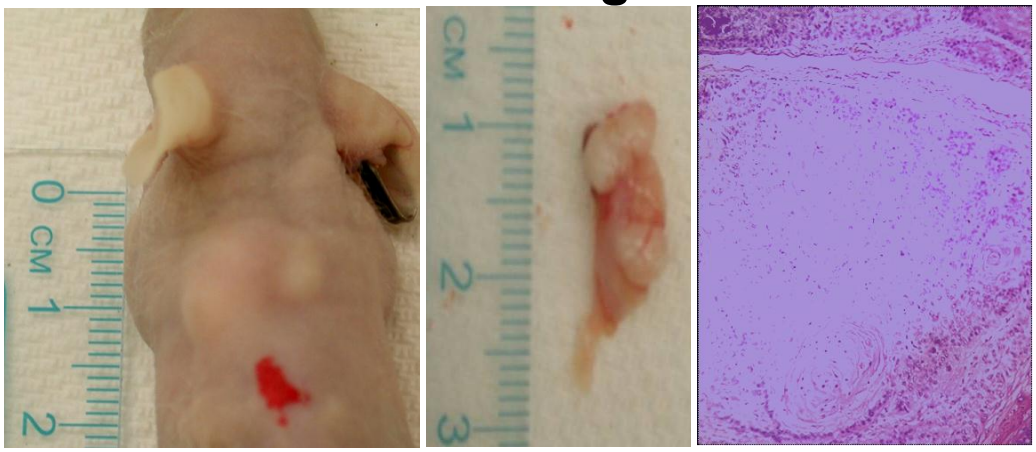
CD133+ SCC can be serially transplanted - demonstrating the stem cell properties of self-renewal and tumor reconstitution

Primary SCC Xenografts



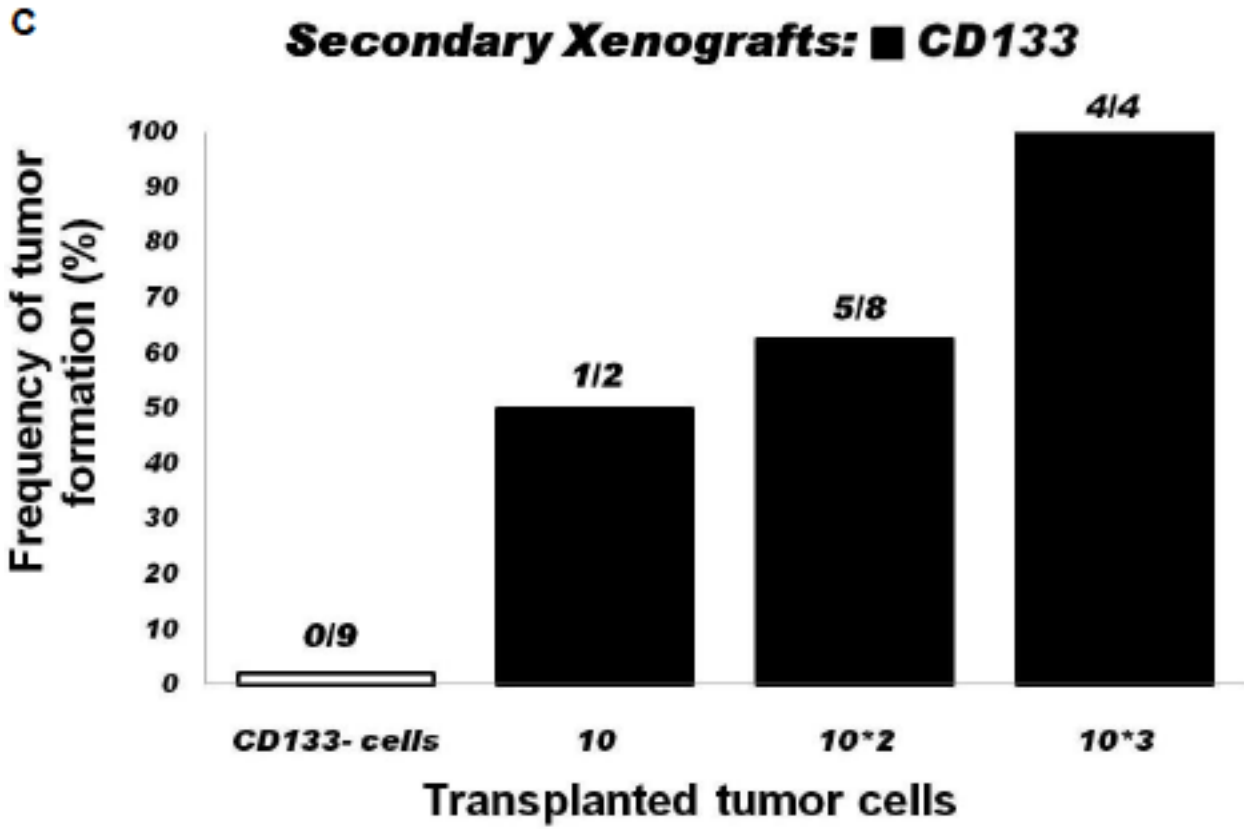
FACS analysis of CD133+ / CD45- cells in primary SCC xenograft = **0.7%** (n=11)

Serial transplants of CD133+ from 1' xenografts



Secondary SCC Xenografts

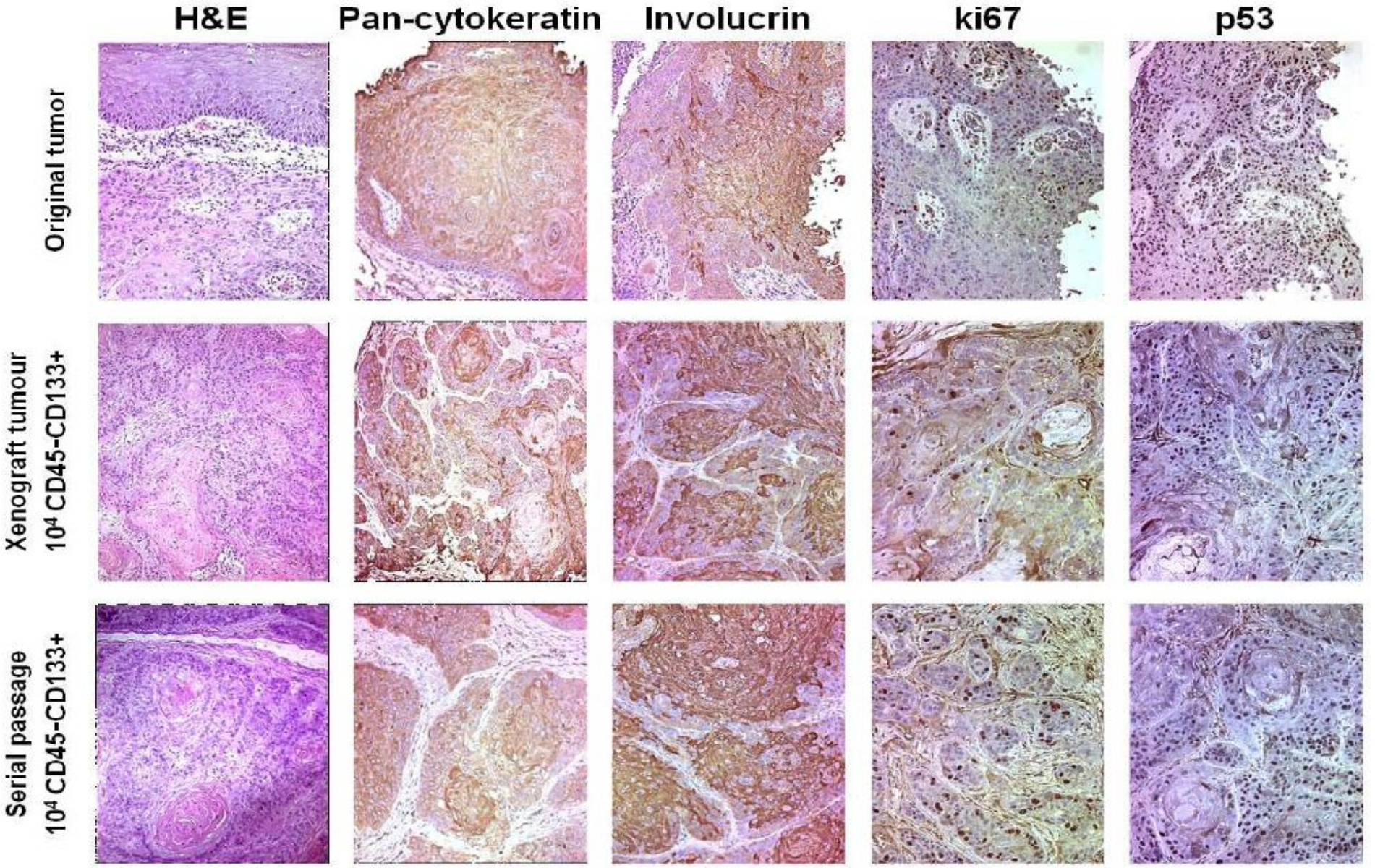
Human CD133+ SCC cells are enriched for TIC when serially transplanted into mice



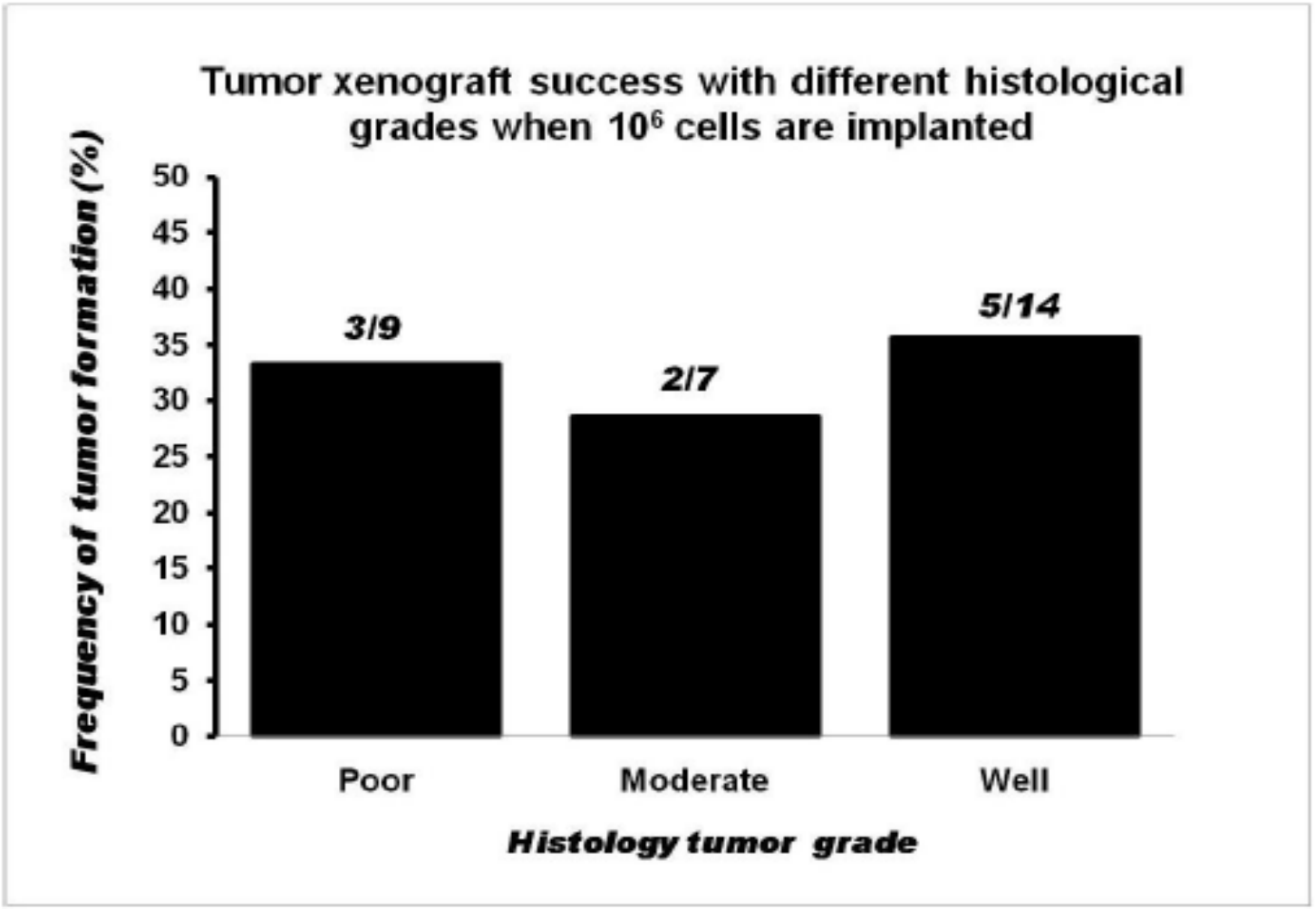
14 total serial xenografts from 8 different human SCC specimens

TIC frequency = 1 / 863

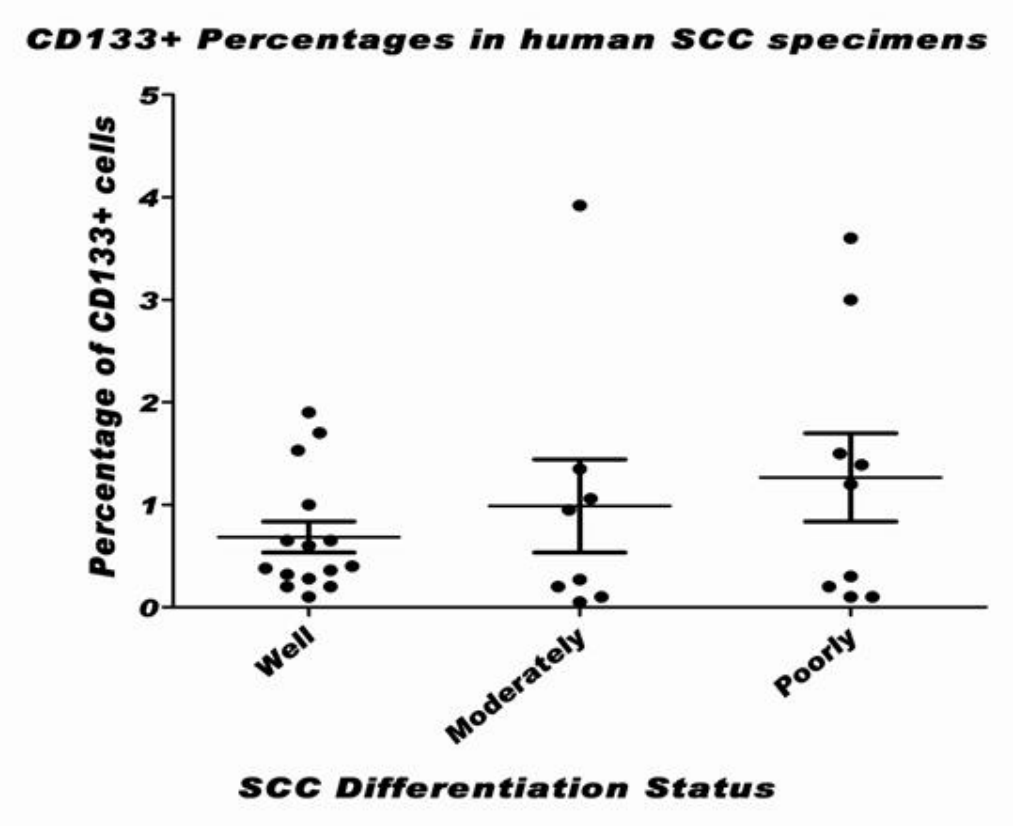
Xenotransplanted CD133+ SCC cells recreates the original tumor morphology



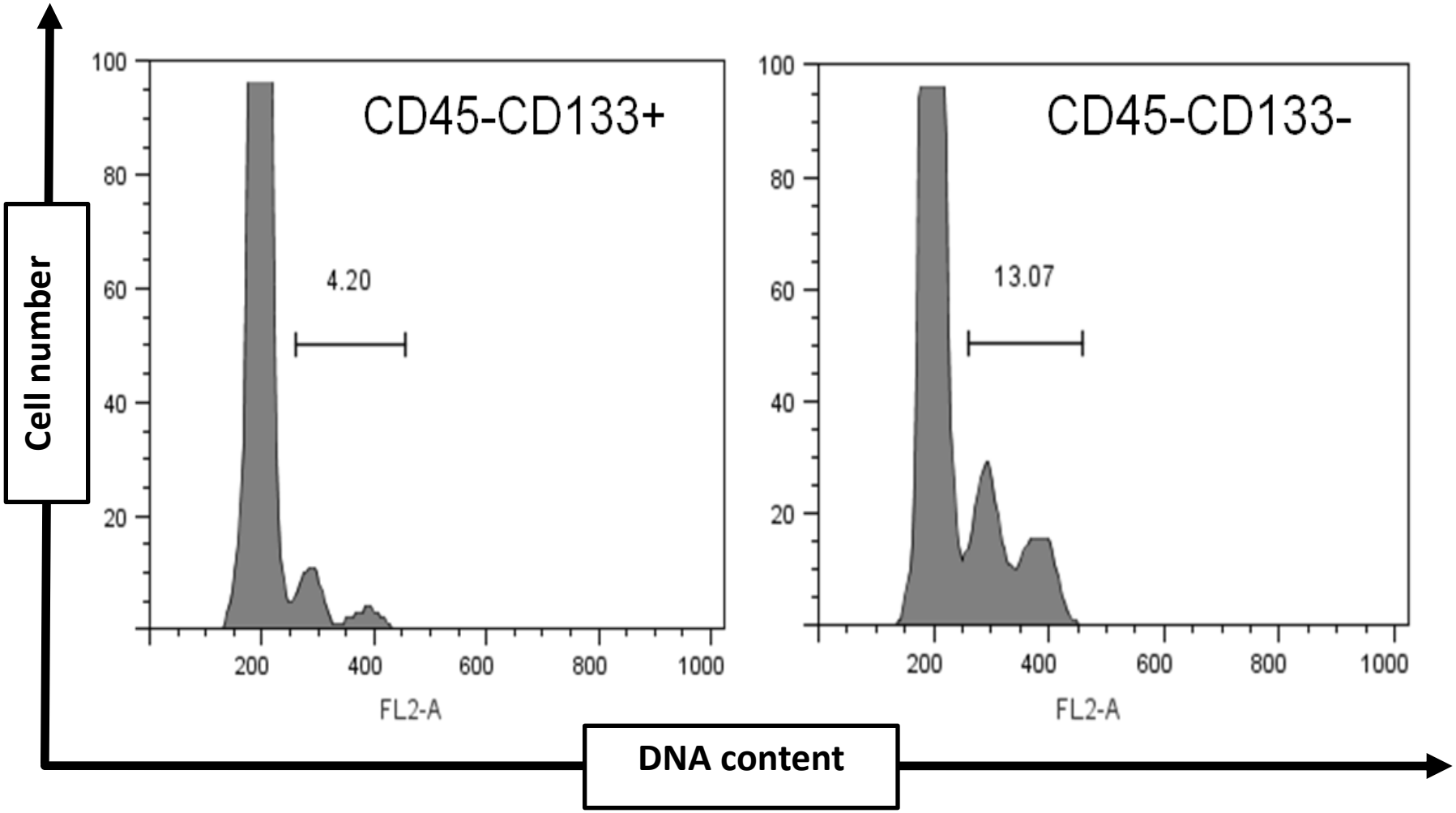
Different SCC histological grades had equivalent rates of tumor formation



Different SCC histological grades had equivalent percentages of CD133+ cells



CD133+/CD45- cells were relatively quiescent



Conclusions

A discrete small sub-population (1%) of human CD133+ SCC cells are highly enriched for tumor-initiating cells (TIC) SCC in an in vivo SCC xenograft model.

The CD133+ cells could recapitulate the histology and hierarchy of the original SCC tumor.

The percentages of CD133+ TIC in SCC tumors of different histological grades were equivalent.

Future Considerations

Future studies will focus on how normal skin developmental programs have been altered in SCC by analyzing TIC for:

- **-Global gene expression profiles**
- **-Genetic and epigenetic changes**
- **-Stromal microenvironment or “niche” influences on TIC behavior**

Other SCC tumors, such as the highly aggressive SCC in renal transplant patients also need to be studied.

Enriched TIC also represent potentially valuable targets for therapeutic strategies that can selectively inhibit their growth and self-renewal.

Acknowledgments

Vogel Lab
Atsushi Terunuma
Jean-Philippe Therrien
Manabu Ohyama
Girish Patel
Renuka Pudi
Carole Yee

NCI
Stuart Yuspa

Dermatology branch
Mark Udey
Maria Turner
Steven Katz

Medicine Branch
William Telford
Veena Kapor

Cancer stem cell interest group -
Barbara Vonderhaar
Snorri Thorgeirsson

Laboratory of Cellular Oncology,
Michael Radonovich
Cindy Masison
John N. Brady

Laboratory of Pathology, CCR
Michael Emmert-Buck