MODELLING THE EFFECT OF SYMMETRICAL DIVISION OF CANCER STEM CELLS ON TUMOUR RESPONSE TO RADIATION

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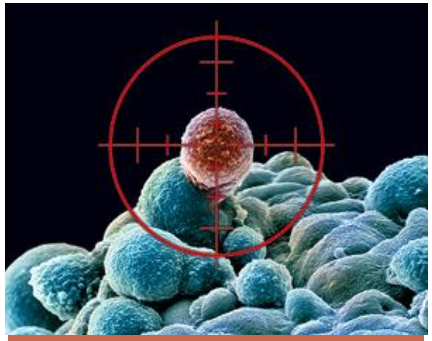


ICTR-PHE 2016

CHALLENGES IN THE MANAGEMENT OF HEAD&NECK TUMOURS*

Clinical challenges:

- preservation of critical organ function
- management of treatment side-effects
- timing of multimodality treatments

Radiobiological challenges:

- tumour hypoxia
- accelerated repopulation of tumour cells during treatment

* L. Marcu & E. Yeoh **2009** *J Cancer Res & Clin Oncol* 135(10):1303-1314

PREDICTIVE ASSAYS FOR TUMOURS

Aim: to choose a treatment protocol that is optimal for each individual patient.

Classification of assays:

- **oxygen status**
- **proliferative potential**
- **intrinsic cellular radiosensitivity**

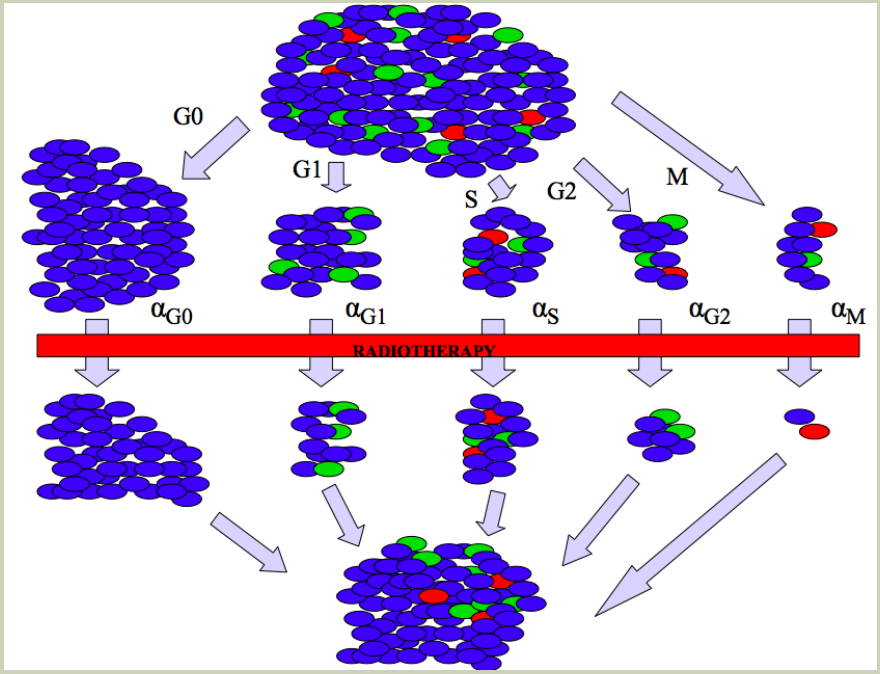
HOWEVER: despite some promising *in vitro* results, the large majority of predictive assays have failed during *in vivo* testing.

E. Hall *Radiobiology for the radiologist* 7th ed – "*... we considered omitting the chapter on predictive assays because they have yet to justify their early promise*".

FROM PREDICTIVE ASSAYS TO *IN SILICO* MODELLING

MODELS:

- allow predictive assessment of the multi-parameter processes that govern radiotherapy outcome
- allow quantitative treatment optimisation
- answer to “what if” questions



L. Marcu *et al* **2002** *Australas Phys Eng Sci Med* 25:155-161

MODELLING AIMS (I)

To grow a virtual HNC with biologically realistic parameters.

To include CSC in the model* and implement their properties based on literature findings.

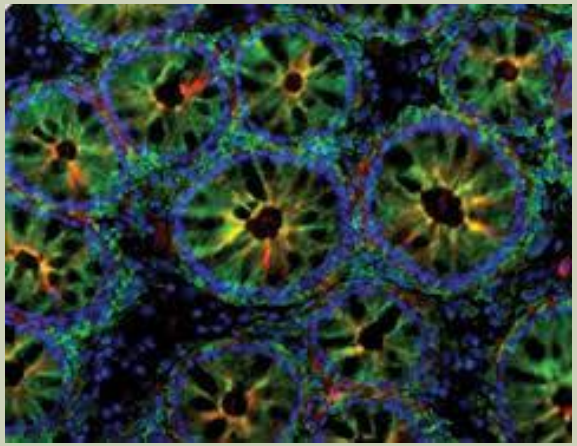
To evaluate the effect of CSC division pattern on tumour response to radiotherapy under hypoxic conditions.

* L. Marcu & E. Bezak **2012** *Cell Prolif* 45(5):404-412

CURRENT TREATMENT CHALLENGES: CANCER STEM CELLS

Cancer stem cells (CSC) are a subpopulation of cells originating from stem cells and have the following properties[1]:

- are long lived,
- have the ability to proliferate indefinitely
- can generate al heterogeneous lineages of the original tumour
- can recreate themselves by symmetric division[2]
- are more radioresistant than non-stem cancer cells[3]
- they preferentially reside in special microenvironmental niches within the tumour[4]



Nature Med 14, 814 (2008) doi:10.1038/nm0808-814

[1] N. Moore et al **2011** *J Oncology* 396076
[2] S. Morrison et al **2006** *Nature* 441, 1068
[3] D. Ramirez-Guerrero **2015** *AAAS* abstract
[4] C. Peitzsch et al **2014** Int J Radiat Biol 90, 636

HEAD & NECK TUMOUR GROWTH MODEL DURING RT

Cell kinetic parameter	Mean value (model)	Mean value & range (literature)	Publication
Volume doubling time	**52 days**	45 days (33-150)	Begg & Steel, 2002
Labelling index	**5%**	7% (5-17)	Steel, 1989
T_S (length of the S phase)	**11 hours**	10.7 hours (4.4-45.7) 11 hours	Begg et al, 1999 Tannock & Hill, 1998
T_C (cell cycle time)	**33 hours (20-60)**	$3xT_S$	Hall, 2000
Tumour composition	▪ **CSC** ▪ **Differentiated cells** ▪ **Quiescent cells**	▪ Cancer stem cells ▪ Cells capable of limited divisions ▪ Non-proliferating cells	Tubiana, 1986 Prince 2007, Moore 2011
Percentage of CSCs in the tumour	5-6% (pre treatment) ? (during treatment)	1.7 – 13.5% (HNC) 12.3% (HNC) 0.4 – 82.7% (other cancers)	Tang 2013 Harper 2007 Huang 2013

RT: CLINICAL JUSTIFICATION

The RTOG 9003 trial has shown that:

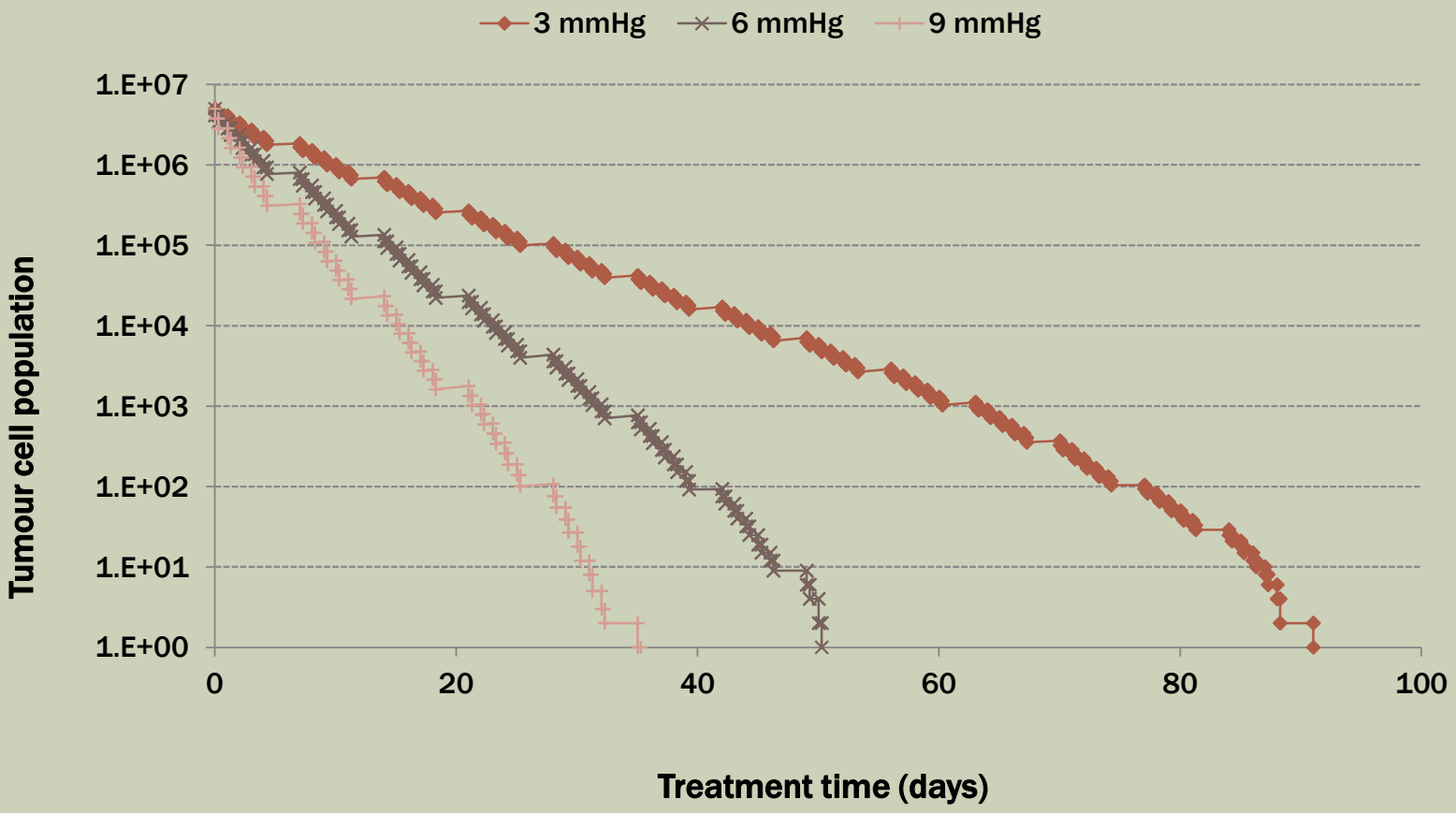
Hyperfractionated radiotherapy for advanced head and neck cancer is the optimal treatment schedule due to the high rate of locoregional control and no significant increase in late toxicity when compared with standard treatment regimens (Fu et al. 2000, Beitler et al. 2014).

Furthermore, the advantage of hyperfractionation over standard and accelerated radiotherapy has been demonstrated by the meta-analysis undertaken by Bourhis et al. (2006).

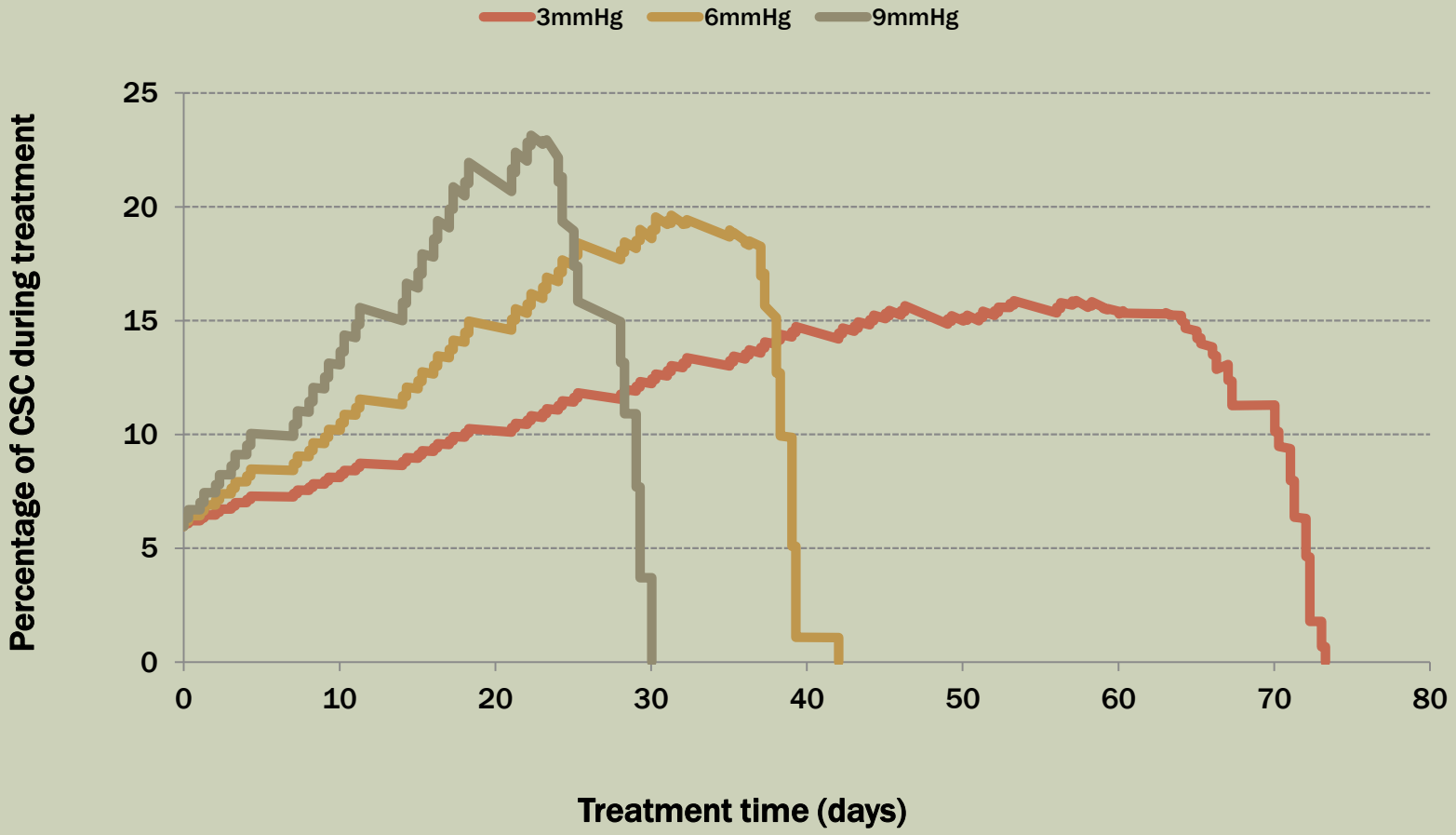
TUMOUR HYPOXIA AND CSC

Experimental findings	References
Hypoxic conditions lead to an increase in the CSC fraction and promote acquisition of stem-like phenotype	*AM McCord et al 2009 Mol Cancer Res 7:489*
VEGF has higher expression in the CSC subpopulation	*S Bao et al 2006 Cancer Res 66:7843*
The presence of HIF2α proteins maintain an undifferentiated phenotype among CSCs (i.e. sustained CSC status)	*A Jogi et al 2002 Proc Natl Acad Sci USA 99:7021*

CELL SURVIVAL CURVES UNDER VARIOUS HYPOXIC CONDITIONS

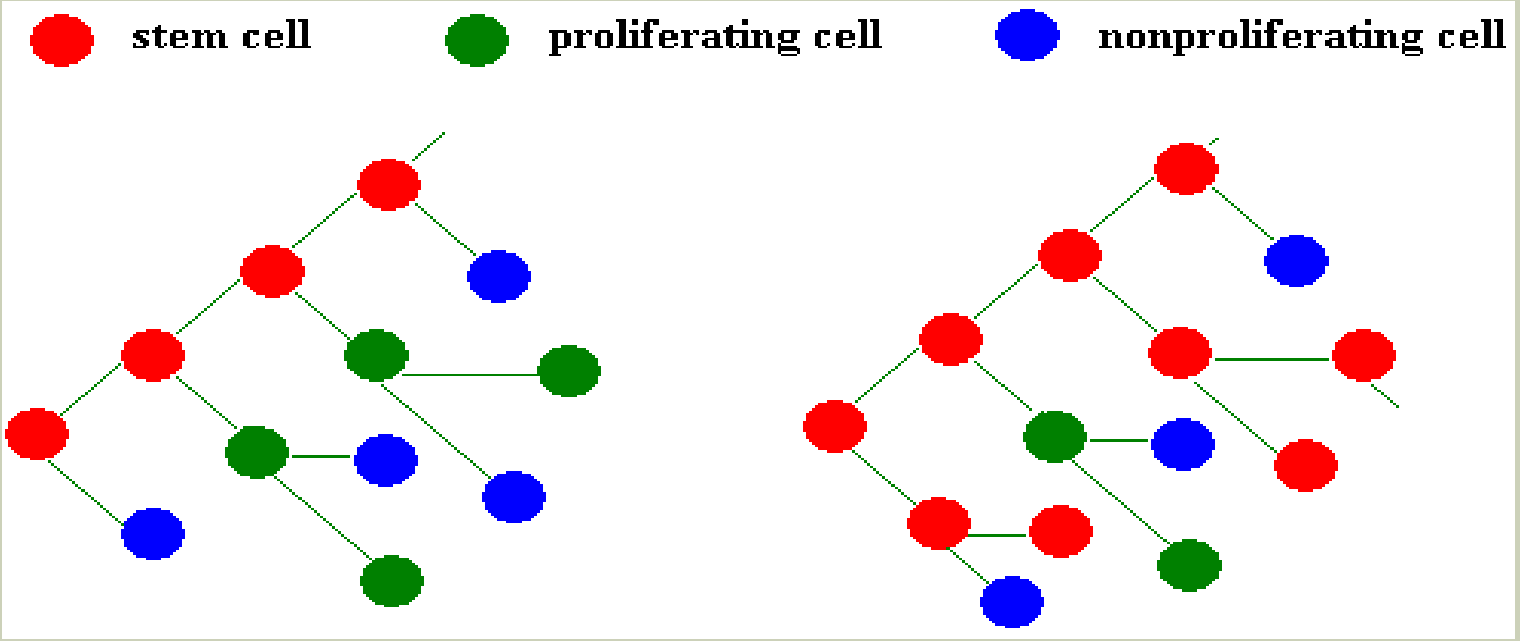


CSC DISTRIBUTION DURING TREATMENT FOR VARIOUS LEVELS OF HYPOXIA



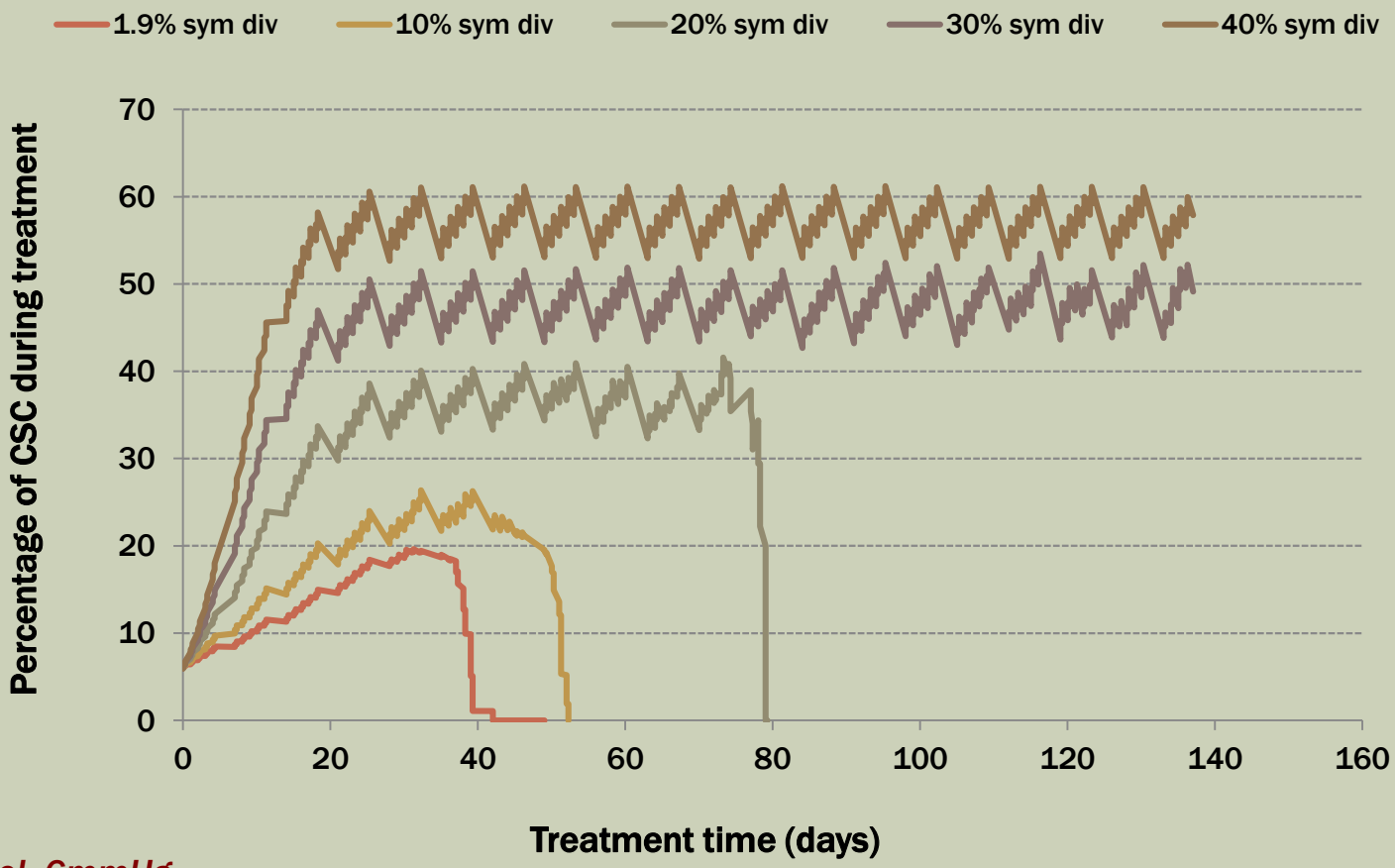
Obs: It nearly appears that hypoxia keeps the CSC subpopulation under control.

CSC DIVISION PATTERN



L. Marcu *et al* **2004** *Phys Med Biol* 49:3676-3779

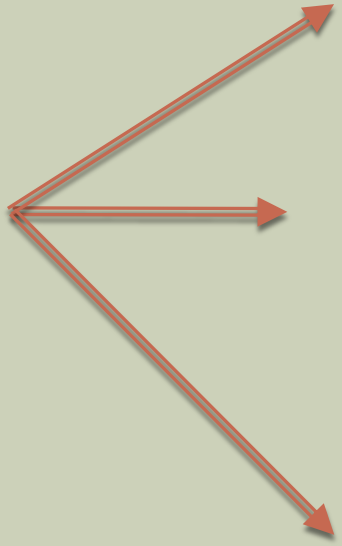
THE IMPACT OF SYMMETRICAL DIVISION PROBABILITY ON CSC SUBPOPULATION



Hypoxic level: 6mmHg

MODELLING AIMS (II)

To evaluate inter-patient variation of tumour response to radiotherapy as a function of:



GROWTH KINETICS

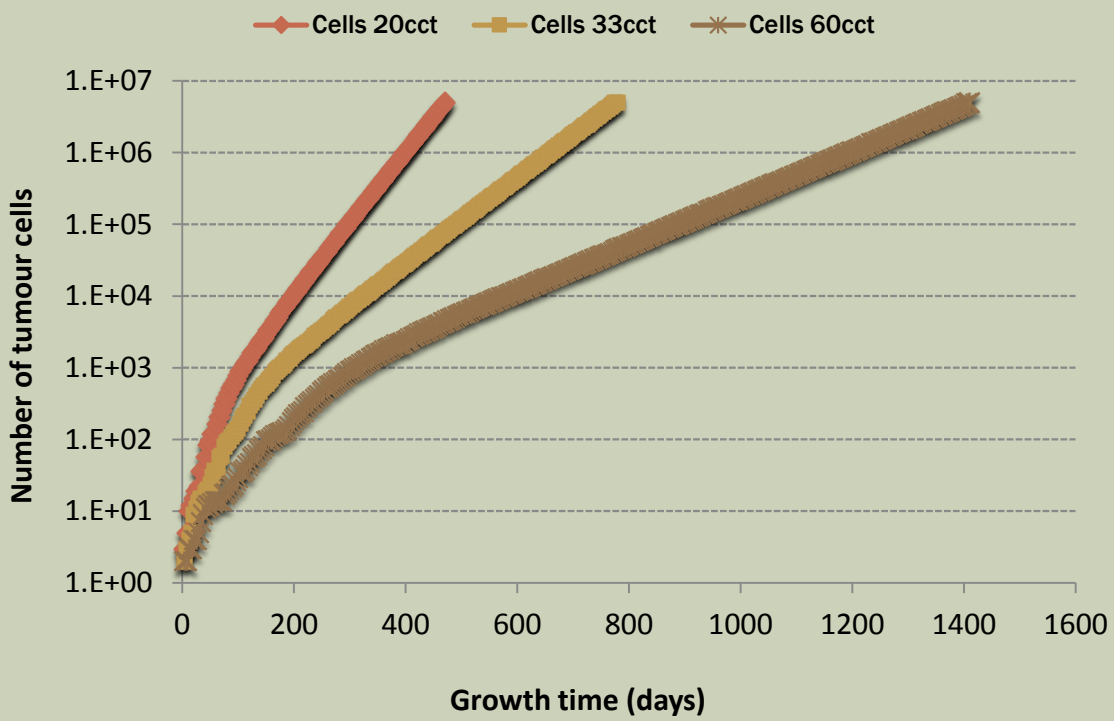
CSC SUBPOPULATION KINETICS

HYPOXIA

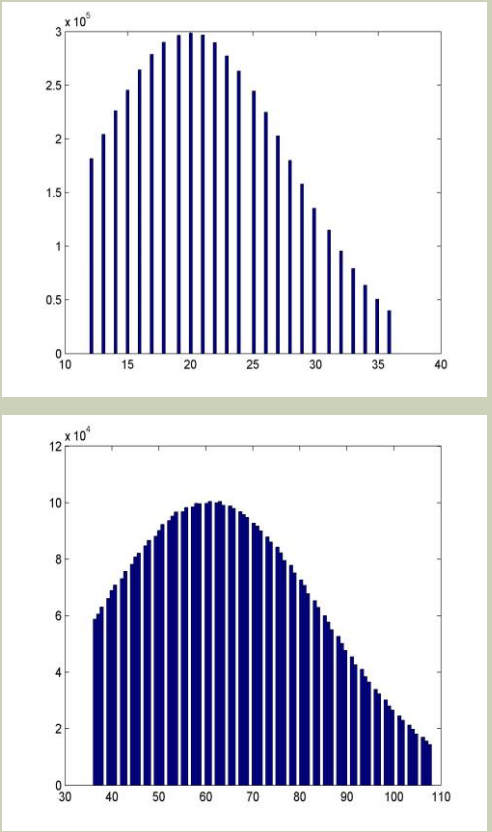
TUMOUR-RELATED PARAMETERS INCLUDED IN THE MODEL

Tumour-related parameters	Values / ranges
Cell cycle time	20h, 33h, 60h
Hypoxia	3mmHg – 10mmHg
Probability of symmetrical division	1.9% - 10%

HEAD AND NECK TUMOUR GROWTH

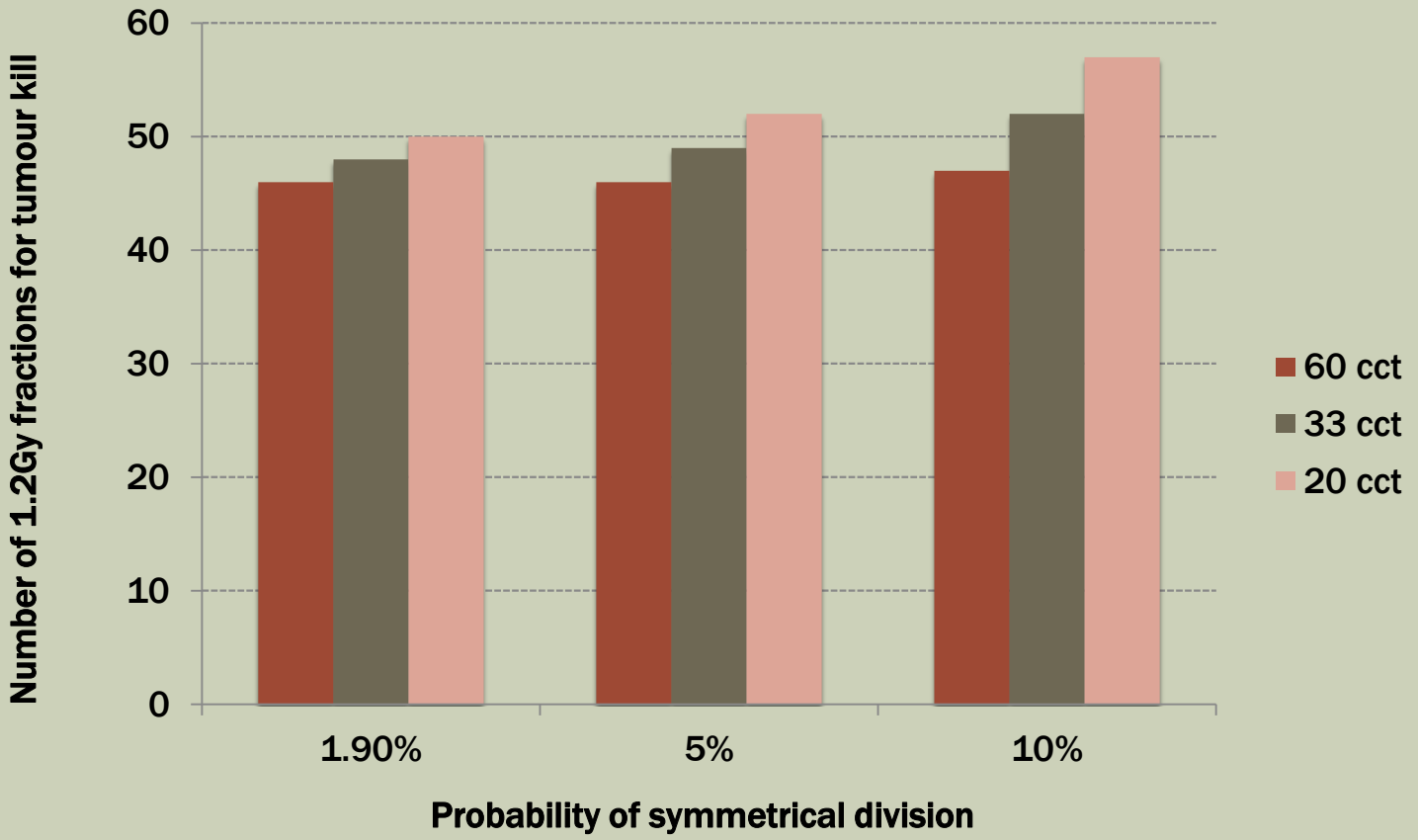


Exponential tumour growth curves for three head and neck cancers with different growth kinetic parameters.



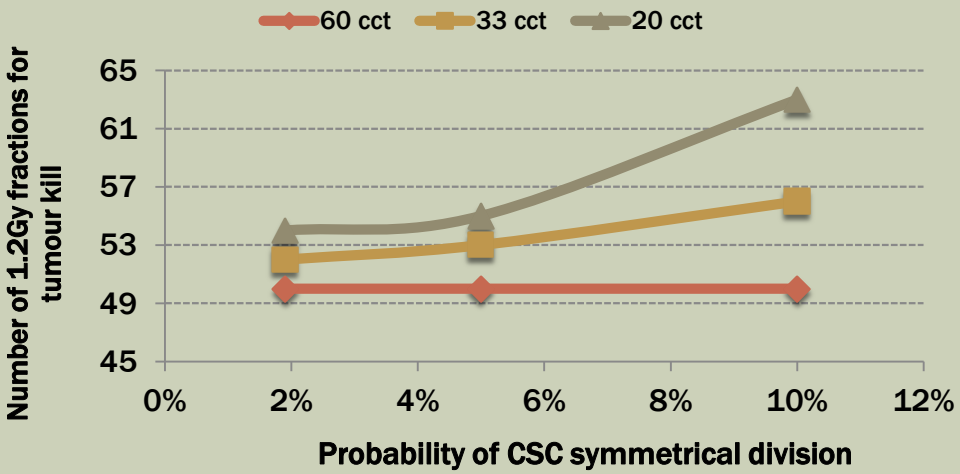
Gaussian distribution of cell cycle times around the mean value

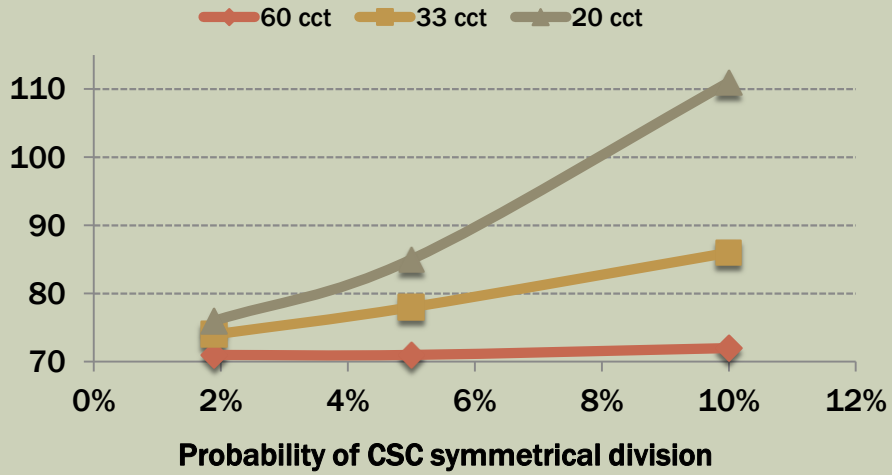
TUMOUR RESPONSE AS A FUNCTION OF GROWTH KINETICS AND CSC DIVISION PATTERN FOR OXIC TUMOURS



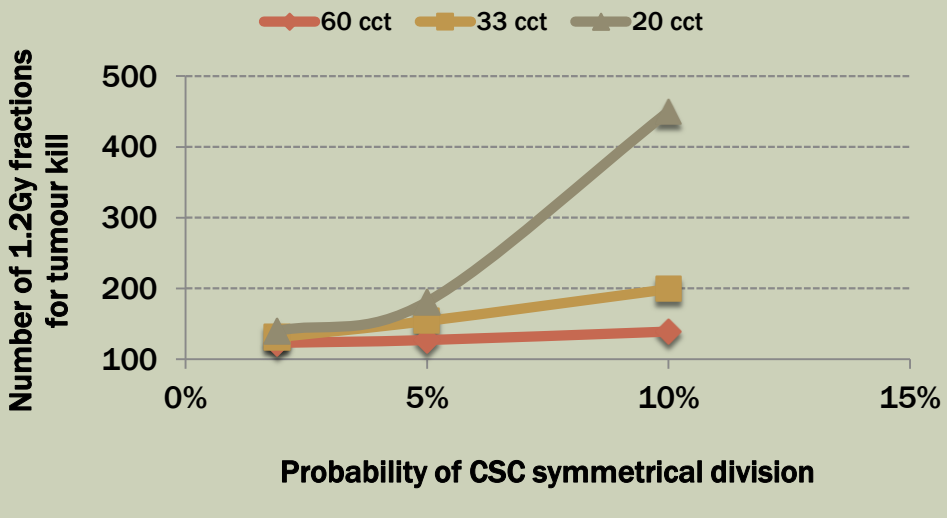
L. Marcu *et al* **2016** *Cell Prolif (in press)*

INTER-PATIENT VARIATION OF TUMOUR RESPONSE TO RT AS A FUNCTION OF CSC DIVISION PATTERN AND TUMOUR HYPOXIA





9mmHg



6mmHg

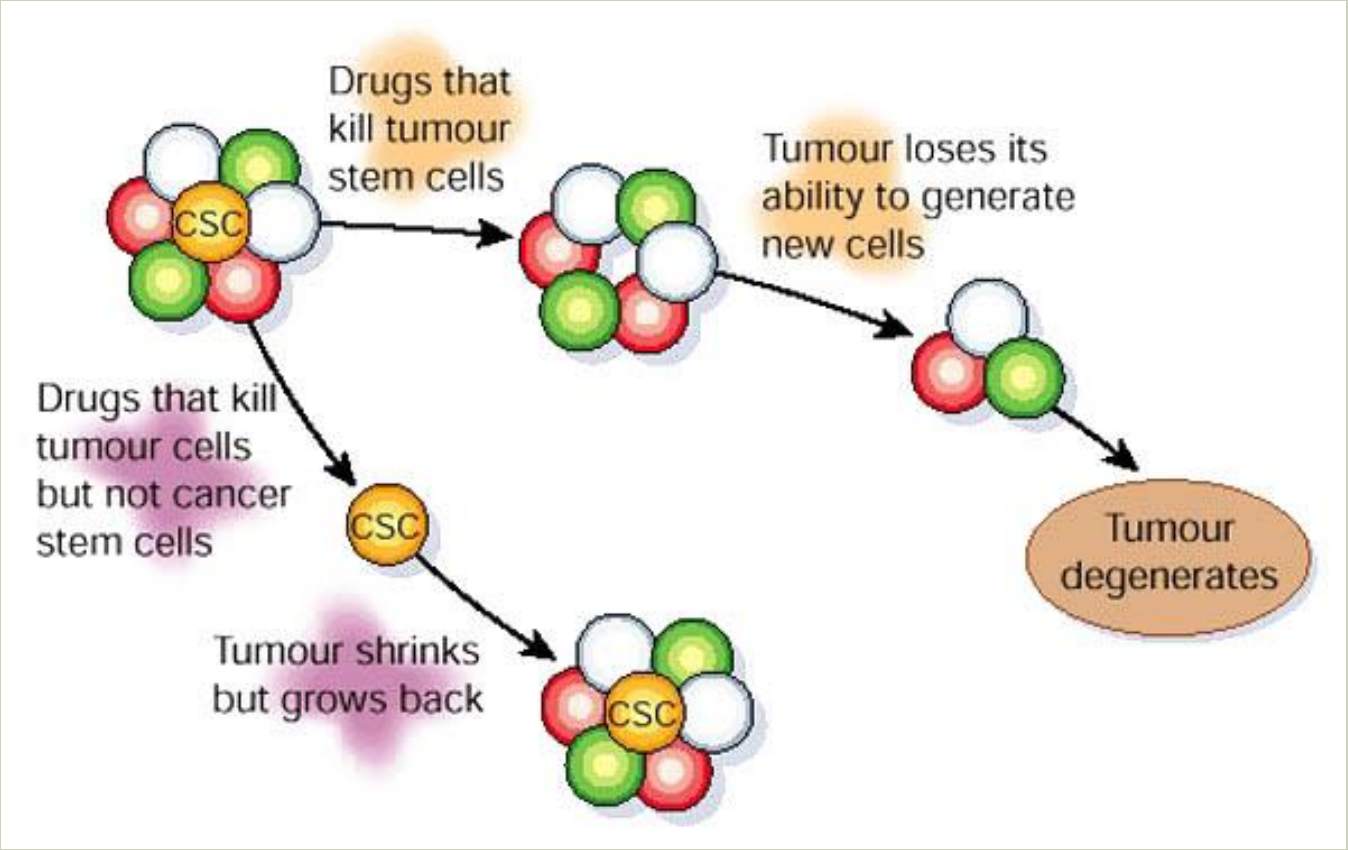
3mmHg

L. Marcu *et al* **2016** *Cell Prolif (in press)*

CONCLUSIONS (I)

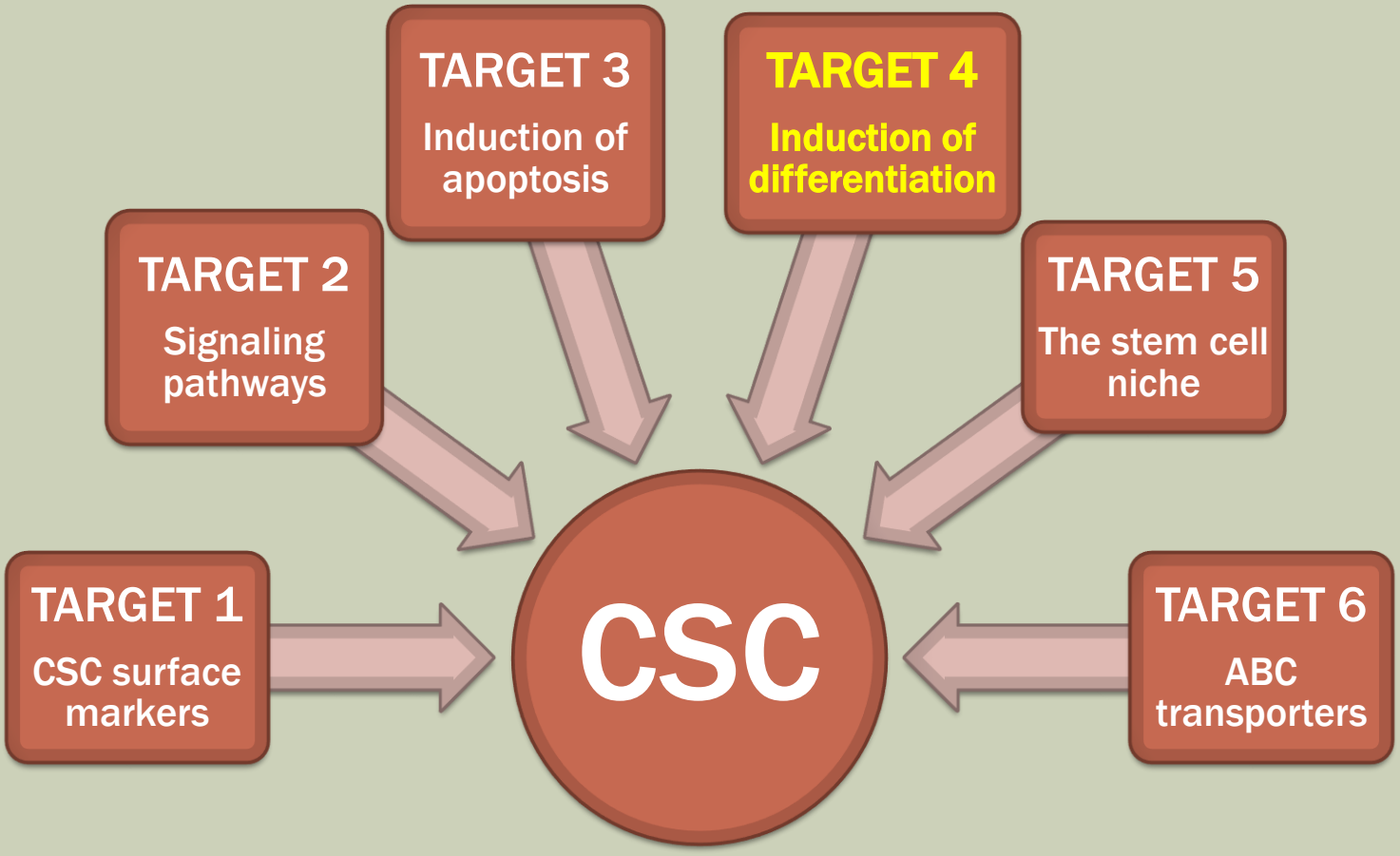
- Oxic/very mildly hypoxic HNC are controlled by hyperfractionated radiotherapy, irrespective of growth kinetics and CSC division pattern.
- Moderately hypoxic tumours show different responses to radiotherapy as a function of growth kinetics.
- In rapidly proliferating tumours the number of fractions needed for tumour control increases exponentially with the probability of CSC symmetrical division, whereas in moderately growing HNC this behaviour is linear.

CSC MARKERS & TREATMENT RESPONSE



R. Tannishtha et al **2001** *Nature* 414, 105-111

CSC - TARGETING



ONGOING AND FUTURE WORK

THEORETICAL LEVEL

- Modelling CSC-targeting through induction of differentiation
- All-trans-retinoic-acid (ATRA)

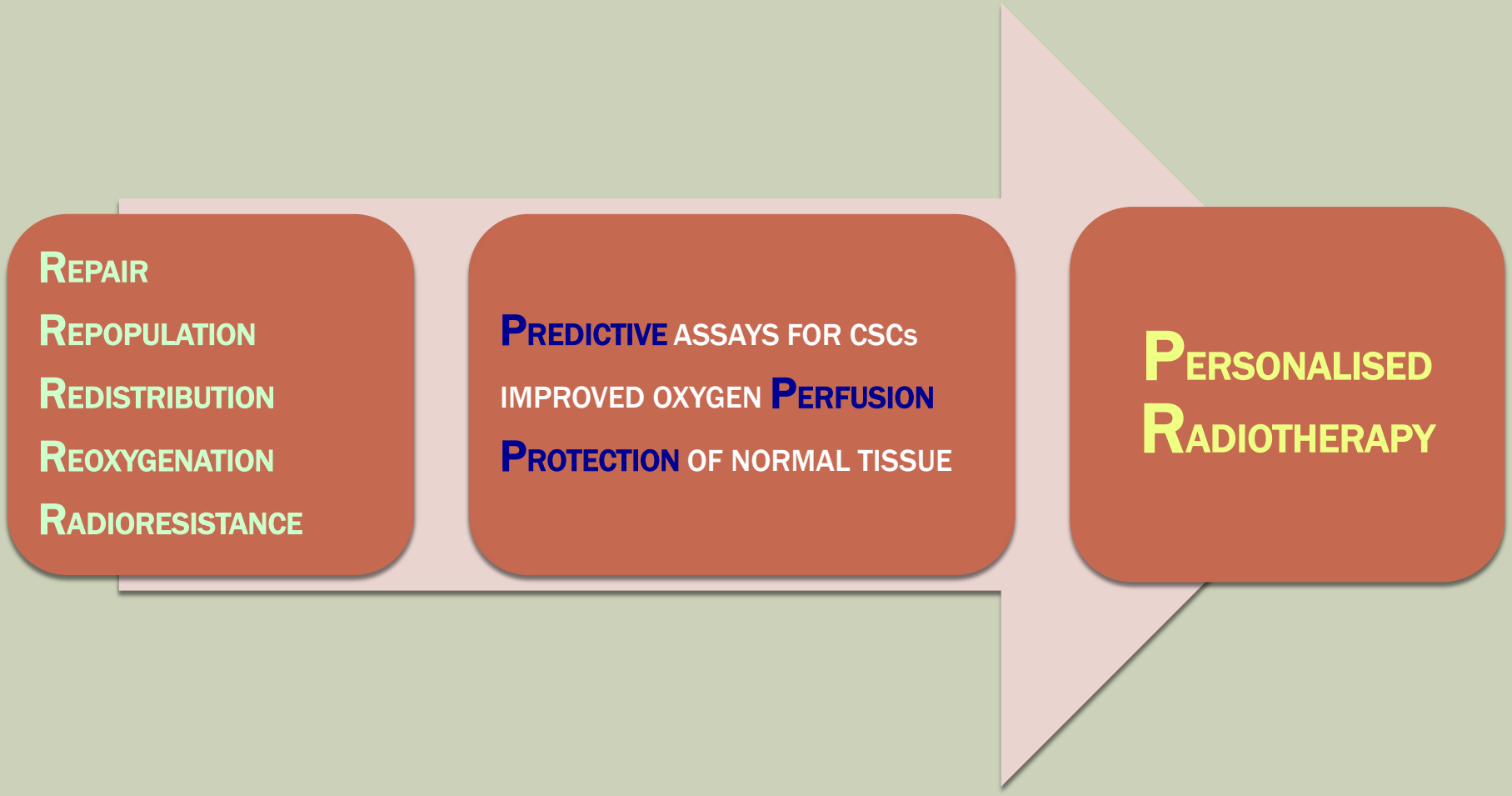
EXPERIMENTAL LEVEL

- HNC cell line growth and CSC identification via surface markers
- Cell line irradiation for determination of SF2

FINAL CONCLUSIONS

- Differences in treatment response of various HNC models due to CSCs and their interplay with growth kinetics show the importance of **CSC identification** within the tumour.
- The need for accurate markers for **CSC labelling** is therefore imminent.
- Both **quantitative and qualitative knowledge on CSC** is needed to describe the resistant subpopulation and to design treatment regimens accordingly.

TOWARDS PERSONALISED TREATMENT IN HEAD AND NECK CANCER



THANK YOU

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