Repopulation concepts in irradiated normal tissues and tumours

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Stem cells — Progenitors/Amplification — Differentiation — Mature/Functional

“Niche”
“Labelling Index is governed mainly by dividing Transit cells, not by the Stem cells”
<table>
<thead>
<tr>
<th>Cancer</th>
<th>Human target cells</th>
<th>Markers</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemia</td>
<td>Haematopoietic stem cells (and potentially some progenitor cells)</td>
<td>CD34⁺, CD59⁺, Thy1⁺, CD38(^{\text{low}})-, C-Kit/low, lin⁻</td>
<td>Endosteal and vascular niches</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Mammary stem cells</td>
<td>Possibly CD24⁺ CD29high, and possibly K6</td>
<td>Mammary MaSC niche</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>Follicular stem cells</td>
<td>Possibly Oct4⁺Pax8⁺Tg⁻</td>
<td>Solid cell nests (SCNs)</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>Mucosal stem cells</td>
<td>Possible LRC (defensin5⁻, Muc2⁻, chromograninA⁻)</td>
<td>Gastric pits, 60–100 µm depth</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>Mucosal stem cells (possibly also some daughter cells)</td>
<td>Lgr5⁺; mTert⁺; possibly also DCAMKL⁻</td>
<td>Crypt base, 280–300 µm depth</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>Possibly Clara, Clara variant, or BASC cells</td>
<td>SCGBa1a, (SP)-C, Sca-1, CD45⁻, CD31⁻; also possibly (c-KIT⁺, Nanog, Oct ¾, KLF4, Sox2) cells</td>
<td>Bronchiolar–alveolar duct junction zone, also possible distal lung niche</td>
</tr>
<tr>
<td>Skin cancer</td>
<td>Epidermal stem cells – BCC (also early progenitors – SCC, late progenitors – papillomas)</td>
<td>α₆briCD71(dim); also β⁶bri/CD71(^{\text{dim}})</td>
<td>Interfollicular basal layer, nominal 70 µm depth</td>
</tr>
<tr>
<td>Bone cancer</td>
<td>Mesenchymal stem cells</td>
<td>CD90, CD73, CD105, and possibly Stro-1, CD106, VCAM-1 CD34-negative</td>
<td>MSC niches, also perivascular</td>
</tr>
<tr>
<td></td>
<td>Haematopoietic stem cells</td>
<td></td>
<td>Bone marrow</td>
</tr>
</tbody>
</table>

ICRP Stem cell report (for public consultation until 10-10-2014): [www.icrp.org](http://www.icrp.org)
Stem-cell self-renewal probability ‘p’

- Cell division gives 2p ‘stem’ cells and 2(1-p) differentiated cells.
- In **steady state** $p=0.50$: asymmetric divisions, stem cell number constant. [N.B. If $p=0.51$, stem cell $T_D = T_C \times 35$, giving hyperplasia]
- In **repopulation**, $p>0.5$: some symmetric divisions.
- Average doubling time ($T_D$) for stem cells $= (T_C \times \ln(2)/\ln(2p))$.
- After $n$ divisions, $(2p)^n$ stem cells in time $(T/T_C)$.

**Bone marrow:**
Lajtha et al 1971

**Epidermis:**
Withers 1967

**Intestine:**
Hendry, 1979

$T_D = 22 \ h$
$p = 0.6$
$T_C = 6 \ h$

$T_D = 24 \ h$

Time Internal Between Doses (Days)

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04-Sep-14
Relative iso-effective dose in 2 Gy fractions (% of single dose EQD2)

• Kick-off time 5-7 days in mouse oral mucosa and skin
• Kick-off time 20-30 days in rat and pig skin
• Recovery/repopulation equivalent to near 1 fraction of 2 Gy per day
• [Other examples of accelerated repopulation in bone marrow and intestine]

W Doerr in Basic Clinical Radiobiology, 2009
A symmetry loss, acceleration, abortive divisions

3 ‘A’s, W Doerr 1997, and in Basic Clinical Radiobiology, 2009
Dose recovered per day owing to repopulation $D_{\text{prolif}}$ from clinical studies

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Endpoint</th>
<th>$D_{\text{prolif}}$ (Gy/day)</th>
<th>95% CL (Gy/day)</th>
<th>$T_k^+$ (days)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early reactions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>Erythema</td>
<td>0.12</td>
<td>$-0.12; 0.22$</td>
<td>$&lt;12$</td>
<td>Bentzen et al. (2001)</td>
</tr>
<tr>
<td>Mucosa</td>
<td>Mucositis</td>
<td>0.8</td>
<td>0.7; 1.1</td>
<td>$&lt;12$</td>
<td>Bentzen et al. (2001)</td>
</tr>
<tr>
<td>Lung</td>
<td>Pneumonitis</td>
<td>0.54</td>
<td>0.13; 0.95</td>
<td></td>
<td>Bentzen et al. (2000)*</td>
</tr>
<tr>
<td>Tumours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head and neck</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larynx</td>
<td></td>
<td>0.74</td>
<td>0.30; 1.2</td>
<td></td>
<td>Robertson et al. (1998)</td>
</tr>
<tr>
<td>Tonsils</td>
<td></td>
<td>0.73</td>
<td>30</td>
<td></td>
<td>Withers et al. (1995)</td>
</tr>
<tr>
<td>Various</td>
<td></td>
<td>0.8</td>
<td>0.5; 1.1</td>
<td>21</td>
<td>Roberts et al. (1994)</td>
</tr>
<tr>
<td>Various</td>
<td></td>
<td>0.64</td>
<td>0.42; 0.86</td>
<td></td>
<td>Hendry et al. (1996)*</td>
</tr>
<tr>
<td>Esophagus</td>
<td></td>
<td>0.59</td>
<td>0.18; 0.99</td>
<td></td>
<td>Geh et al. (2005)</td>
</tr>
<tr>
<td>Non-small cell lung</td>
<td></td>
<td>0.45</td>
<td>N/A</td>
<td></td>
<td>Koukourakis et al. (1996)</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td></td>
<td>0.52</td>
<td>0.29; 0.75</td>
<td>0 or 21</td>
<td>Hinata et al. (2001)</td>
</tr>
</tbody>
</table>

- Human oral mucosa: Kick-off time at $<12$ days
- $D_{\text{prolif}} \sim 0.8$ Gy per day (not much different for Head & Neck tumours)

*S Bentzen and M Joiner, in Basic Clinical Radiobiology, 2009*
Tumour Cell Kinetics
\( T_{pot}: \) Potential doubling Time

Cell cycle time

\( T_c \)

2 days

Growth Fraction

50-40%

Potential doubling time

\( T_{pot} \)

4-5 days

Cell Loss Factor

90%

Volume doubling time

\( T_{vol} \)

40-60 days

\[
T_{pot} = \frac{T_c}{GF} = \frac{T_s}{LI} \quad T_{vol} = \frac{T_{pot}}{1-CLF}
\]

Steel 2002
Fowler 1991
2 Gy Dose fractions

Tumour volume

\[ T_{\text{vol}} = T_{\text{clon}} = 40-60 \text{d} \]

without radiation

Total cells

No. of cells

100

10

1

Time

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04-Sep-14

Td Clon/CSC

Clon/CSC

T_d Clon/CSC
Tumour repopulation: avoid treatment interruptions

- More dose required to control tumours with increases in overall treatment time.
- Loss of local control with gaps in treatment - 7 to 10% per week for Head & Neck SCC, equivalent to 0.6 Gy per day using 2 Gy fractions.
- Avoid treatment interruptions, or compensate for 1 day gap best by 6F/week or 2 fractions (8 hours apart) on Friday.
- These guidelines have saved lives.

J Hendry, S Bentzen, R Dale, J Fowler, T Wheldon, B Jones, A Munro, N Slevin, A Robertson (1996)
Five versus six fractions of radiotherapy per week for squamous-cell carcinoma of the head and neck (IAEA-ACC study): a randomised, multicentre trial (2010)

Jens Overgaard, Bidhu Kaylan Mohanti, Naseem Begum, Rubina Ali, Jai Prakash Agarwal, Maire Kuddu, Suman Bhasker, Hideo Tatsuzaki, Cai Grau

Shortening treatment by 1 week improved outcome

04-Sep-14

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A randomized multicentre trial of Accelerated Hypo- vs. Normo-fractionated radiotherapy for head & neck squamous cell carcinoma (HYPNO trial)

International Atomic Energy Agency - 2014 Clinical Research Project –

Soeren Bentzen – Baltimore Agency staff.
Centres in Argentina, Brazil, Cuba, India, Indonesia, Pakistan, Philippines, South Africa, Thailand, Macedonia, Uruguay,
Head & Neck scc: lineage status?

Marginal - basal/suprabasal

Intermediate - basal/deeper

Random

Mixed - marginal/random

Histology courtesy of G Wilson
Tumour differentiation status

Well-differentiated scc

• High cell-loss factors
• Greater propensity for accelerated repopulation during XRT
• Should benefit from accelerated XRT

Poorly-differentiated scc

• Low cell-loss factors
• If low LI, use conventional protracted XRT


“…..differentiated tumours are more similar to normal epithelial tissues in their capacity to respond to damage by accelerated repopulation. …response capacity, rather than undisturbed proliferation rate, could be the important factor and a predictor of repopulation ability.”

Adrian Begg (2012)
EGFR and tumour differentiation and the response to accelerated radiotherapy of squamous cell carcinomas of the head and neck in DAHANCA 6 and 7

- EGFR positive tumours responded better to moderately accelerated radiotherapy than those with low EGFR.
- A similar pattern was seen, stratifying by well / moderate vs poor tumour differentiation.
- A combined parameter was constructed:
  - High EGFR, well / moderate differentiation benefited from moderately accelerated radiotherapy - HR 0.54 (0.37-0.78)
  - Low EGFR and / or poor differentiation - HR 0.8 (0.51-1.25)
- Eriksen, Steiniche, Overgaard et al 2005; Radiother Oncol 74: 93-100
All tumour cells malignant? – now Cancer Stem Cells?

W Mackillop, A Ciampi, J Till, R Buick, 1983
Hans Kummermehr

Unirradiated tumour

Dose level

Surviving cells after radiation

Radiation

Non-stem cell
Cancer stem cell
No tumour cells left

Tumour control probability (%)

Radiation dose

Tumour control probability (%)

Permanent local tumour control

AT 17/7 tumour

Homogenous dose distribution assuming random distribution of cancer stem cells over tumour

Standard radiation dose

Increased radiation dose

Niche protecting cancer stem cells from radiation or accumulation of cancer stem cells

Heterogenous ‘dose painting’ with escalated doses

Cancer stem cell

M Baumann, M Krause, R Hill, 2008
### Cancer stem cell (CSC) markers in common cancers

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>CSC marker</th>
<th>CD133</th>
<th>CD44</th>
<th>ALDH1</th>
<th>α2β1 integrin</th>
<th>α6 integrin</th>
<th>CD24</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioma</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[119–123]</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>[6,124–126]</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>[127–131]</td>
</tr>
<tr>
<td>HNSCC</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>[21,132]</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>[133–136]</td>
</tr>
</tbody>
</table>

**Protein family**
- Transmembrane glycoproteins
- Transmembrane glycoprotein, hyaluronic acid receptor
- Aldehyde dehydrogenase
- Heterodimeric transmembrane glycoprotein receptors
- Adhesion, migration, invasion, CXCR4 signaling

**Function in CSC**
- Endocytosis, co-regulation of growth factors, PI3K pathway activation, self-renewal
- Adhesion, co-regulation of growth factors EMT, STAT3 signaling activation, migration, drug resistance, self-renewal
- Aldehyde detoxification, drug resistance, oxidative stress response
- ECM interaction, cell–cell interaction, cytoskeletal rearrangement, co-regulation of growth factors, signal transduction
- Adhesion, migration, DNA damage-induced nuclear factor-kappaB (NF-κB) signaling

**References**
- [137–140]
- [141–145]
- [146]
- [147]
- [148–150]

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*C Peitzsch, I Kurth, L Kunz-Schughart, M Baumann, A Dubrovska, 2014*
Clinical studies: correlation between CSC markers and radiotherapy outcome

<table>
<thead>
<tr>
<th>Entity</th>
<th>Marker</th>
<th>Outcome</th>
<th>Prognostic correlation</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioblastoma</td>
<td>CD133CD15</td>
<td>Overall survival</td>
<td></td>
<td>Kim et al. [32]</td>
</tr>
<tr>
<td></td>
<td>Nestin</td>
<td>Progression-free survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 88</td>
<td>CD133</td>
<td>Overall survival</td>
<td>+</td>
<td>Murat et al. [33]</td>
</tr>
<tr>
<td>n = 80</td>
<td>CD133</td>
<td>Overall survival</td>
<td>+</td>
<td>Pallini et al. [34]</td>
</tr>
<tr>
<td>n = 44</td>
<td>CD133</td>
<td>Progression-free survival</td>
<td>+</td>
<td>Metellus et al. [118]</td>
</tr>
<tr>
<td>n = 48</td>
<td>CD133</td>
<td>Overall survival</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Progression-free survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HNSCC</td>
<td>CD44</td>
<td>Local tumour control</td>
<td>+</td>
<td>de Jong et al. [35]</td>
</tr>
<tr>
<td>n = 52</td>
<td>CD44 Integi-β1</td>
<td>Local-progression-free survival</td>
<td></td>
<td>Koukourakis et al. [36]</td>
</tr>
<tr>
<td>n = 74</td>
<td>CD44</td>
<td>Metastases-free survival</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oesophageal cancer</td>
<td>CD44+/CD24-</td>
<td>Pathological tumour response</td>
<td>+</td>
<td>Smit et al. [36]</td>
</tr>
<tr>
<td>n = 24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectal cancer</td>
<td>CD133</td>
<td>Disease-free survival</td>
<td>+</td>
<td>Wang et al. [41]</td>
</tr>
<tr>
<td>n = 73</td>
<td></td>
<td>Overall survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 210</td>
<td>CD44v6</td>
<td>Disease-free survival</td>
<td>+</td>
<td>Avoranta et al. [119]</td>
</tr>
<tr>
<td>n = 99</td>
<td>CD133</td>
<td>Disease-specific survival</td>
<td>+</td>
<td>Sprenger et al. [40]</td>
</tr>
<tr>
<td>n = 52</td>
<td>CD133CD44</td>
<td>Cancer-specific overall survival</td>
<td>+</td>
<td>Kawamoto et al. [42]</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>CD24</td>
<td>Metastases-free survival</td>
<td>+</td>
<td>Kwon et al. [38]</td>
</tr>
<tr>
<td>n = 73</td>
<td></td>
<td>Locoregional failure rate</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>n = 140</td>
<td>CD24</td>
<td>Metastases-free survival</td>
<td>+</td>
<td>Sung et al. [39]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Locoregional failure-free survival</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall survival</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*R Butof, A Dubrovska, M Baumann, 2014*
**EGFR**

- regulates cell proliferation (and cell death)
- overexpressed or mutated in cancer
  - associated with adverse prognosis
  - associated with resistance to cytotoxic therapy

<table>
<thead>
<tr>
<th>Tumour Type</th>
<th>% Expressing</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC</td>
<td>40-80%</td>
</tr>
<tr>
<td>Renal Carcinoma</td>
<td>50-90%</td>
</tr>
<tr>
<td>Breast</td>
<td>14-91%</td>
</tr>
<tr>
<td>Ovarian</td>
<td>35-70%</td>
</tr>
<tr>
<td>Glioma</td>
<td>40-50%</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>30-50%</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>80-100%</td>
</tr>
<tr>
<td>Colon</td>
<td>25-77%</td>
</tr>
<tr>
<td>Bladder</td>
<td>31-48%</td>
</tr>
</tbody>
</table>
EGFR expression is prognostic for the outcome of RT in H&N SCC

Ang et al., Cancer Res 62: 7350-7356, 2002
Independent and functional validation of a multi-tumour-type proliferation signature. *Starmans et al.* (2012) *BJC* 107, 508. “We have developed and validated a qPCR-based proliferation signature. This test might be used in the clinic to select (early-stage) patients for specific treatments that target proliferation.”

**Imaging proliferation - PET**

- Positron emission tomography probes for proliferation: $^{18}$F-FLT, $^{11}$C-thymidine, $^{11}$C-FMAU
- $^{18}$F-FDG: indirect probe lacking specificity, can reflect tumour number, flow, glucose transport (Glut-1), glycolysis, proliferation, hypoxia
- $^{11}$C-Methionine: reflects proliferation used in brain tumours because of the high uptake of FDG in normal brain

*Supplied courtesy of C West*
Conclusions

- Stem cell markers characterised in several normal tissues.
- Normal tissue repopulation after radiation starts early, and time factor established for some tissues.
- For tumour radiotherapy, avoid treatment interruptions.
- H&N radiotherapy can be accelerated with good outcome.
- EGFR is a useful biomarker of tumour cell proliferation - PCR signature and PET imaging being developed.
- Cancer Stem Cell (CSC) markers characterised in several tumour types. The markers differ from those in the normal tissues of origin.
- The important question remains: Will measurements of CSC improve over those for total cells regarding tumour response to fractionated radiotherapy?
References