Basic radiobiology: fractionation, 5 Rs, $\alpha/\beta$ ratio, QUANTEC

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Radiotherapy (RT) Fractionation

- RT is an important component of multi-modality cancer treatment
  - used in approximately 60% of patients treated with curative intent
- In the 1930’s, RT pioneers discovered that splitting radiation dose into a number of small fractions yielded better outcomes than a single exposure
- RT schedule depends on cancer type & surrounding normal tissue tolerance

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total radiation dose</td>
<td>$D_{\text{total}}$</td>
</tr>
<tr>
<td>Dose per fraction</td>
<td>$D_{\text{frac}}$</td>
</tr>
<tr>
<td>Number of fractions</td>
<td>$N_{\text{frac}}$</td>
</tr>
<tr>
<td>Overall treatment time</td>
<td>$T_{\text{total}}$</td>
</tr>
<tr>
<td>Volume irradiated</td>
<td>$V_{\text{irr}}$</td>
</tr>
</tbody>
</table>

Therefore RT schedules are designed to

- maximize tumour kill
- minimize normal tissue damage

\[
\text{Therapeutic ratio} = \frac{D_{\text{frac}} \times N_{\text{frac}}}{V_{\text{irr}}}
\]
Aims of RT: the therapeutic ratio

Best chance of cure, with least normal tissue side effects

Barnett GC. et al, Nat Rev Cancer 2009
Radiotherapy adverse effects

Acute effects

- Occur during or shortly after RT
- Affect rapidly-proliferating tissues due to cell death
  - E.g. skin, rectum
- Tend to cause inflammation
- Generally manageable
- Usually reversible due to proliferation & repopulation by surviving stem cells

Late effects

- Occur months to years after RT
- Can be permanent
- Wide individual variation in amount and severity of late effects after RT
- 5–10% patients have marked toxicity
- Serious late side effects impact negatively on quality-of-life of cancer survivors
  - Debilitating (e.g. bowel incontinence)
  - Life-threatening (e.g. bowel obstruction)
The 5 Rs of Radiobiology

• Initially described to provide a means of understanding the success or failure of radiotherapy $^{1,2}$

• Relevant to both tumour and normal cells
  • Repair
  • Repopulation
  • Redistribution
  • Reoxygenation
  • Radiosensitivity

• Provide framework to examine new therapeutic strategies from point of view of both tumour and normal cells
  • Enabled development of safe and effective dose-fractionation regimens

1 Withers HR Adv Radiat Biol 1975
2 Steel GG IJROBP 1989
Principal damaging effects of ionizing radiation is due to its ability to eject (ionize) electrons from molecules within cells.

Most biological damage is done by the ejected electrons:
- which go on to cause further ionizations in molecules with which they collide
- progressively slowing down as they go

At the end of e- tracks, interactions with other molecules become more frequent, giving rise to clusters of ionization

- These clusters are a unique characteristic of IR
- Many ionizations can occur within a few base pairs of DNA
- Difficult for cell to deal with damage clusters
How much damage needs to be repaired?

- 1 Gy of RT causes approximately
  - $10^5$ ionizations
  - >1000 damages to DNA bases
  - 1000 single strand breaks
  - 20-40 double strand breaks (DSBs)

- Kills approximately 30% of human cells, as a consequence of efficient DNA repair.
Repair and DNA Damage Response (DDR)

- Complex series of pathways (DDR) for ensuring that DNA remains intact in the face of internal and external attack
- Effectors of DDR: determine the outcome for the cell
  - Programmed cell death pathways: apoptosis
  - DNA repair pathways
  - Damage checkpoints that cause temporary or permanent blocks in progress of cells through the cell cycle
    - E.g. mitotic delay allows repair of DNA damage in cells prior to undergoing DNA replication or mitosis
    - Preventing acquisition of genetic instability in future cell generations
- In clinical courses of fractionated RT, fractions are given daily allowing sufficient time for repair to occur

Hein et al Int J of Oncology
# DNA Damage Repair Pathways

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Target</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base excision repair (BER)</td>
<td>Damaged bases</td>
<td></td>
</tr>
<tr>
<td>Single Strand Break Repair (SSBR)</td>
<td>Single strand breaks</td>
<td>Similar to BER</td>
</tr>
<tr>
<td>Homologous Recombination (HR)</td>
<td>Double strand breaks</td>
<td></td>
</tr>
<tr>
<td>Non-homologous end-joining (NHEJ)</td>
<td>Double strand breaks</td>
<td></td>
</tr>
<tr>
<td>Mismatch Repair (MMR)</td>
<td>Correcting mismatches of bases in DNA</td>
<td>Less relevant to RT</td>
</tr>
<tr>
<td>Nucleotide Excision Repair</td>
<td>Repairing bulky lesions or DNA adducts formed e.g. by UV light or cisplatin</td>
<td>Less relevant to RT</td>
</tr>
</tbody>
</table>

Reviewed in Shibata and Jeggo  Clin Onc 2014
Repopulation

• Each fraction of RT results in a decrease in the number of surviving clonogenic tumour cells
  • i.e. cells that have the ability to repopulate

• Clonogenic tumour cells that survive irradiation can repopulate the tumour by increasing their rate of proliferation and/or reduced cell loss
  ➢ Thereby reduce efficacy of RT
  ➢ Rapid repopulation of clonogenic tumour cells is important in treatment resistance

• **Time factor of radiotherapy**

If tumour can repopulate, increasing overall treatment time will result in more clonogenic cells that need to be killed
  ➢ And therefore a higher dose to achieve local control
Repopulation

- Repopulation is important in
  - tumours whose stem cells are capable of rapid proliferation
  - acutely responding normal tissues, e.g. skin, GI tract, oral mucosa

- In humans accelerated repopulation tends to occur 2-4 weeks after the start of RT

- Repopulation has little consequence in late-responding, slowly proliferating tissues, e.g. kidney
  - do not suffer much early cell death and do not produce an early proliferative response to RT
Evidence for repopulation during fractionated RT for Head and Neck (H&N) cancer: Withers 1988

- Reviewed published studies using various fractionated schedules
- Total dose required to give 50% control of H&N tumours plotted against time of RT
- For overall time less than about 3-4 weeks there is little change in dose required for 50% tumour control
- At longer times: substantial increase in total dose required as treatment time increases

Withers et al 1988
Repopulation in Squamous cell cancer of Head and Neck (H&N)

- Treatment times longer than 3-4 weeks
  - Effect of proliferation equivalent to loss of dose of about 0.4-0.8 Gy a day
- Loss of local control with treatment gaps
  - Gaps compensated by giving 2 fractions per day
    - >6 hours apart to allow repair of normal tissues
  - Evidence for the benefit of accelerated RT schedules

Repopulation, occurring at 2-4 weeks after start of RT, is due to increase in proliferation of clonogenic cells and results in decreased
- Local control of cancer cells
- Acute toxicity
Redistribution

- Radiosensitivity of cells varies considerably as they pass through cell cycle
- Cells in S phase (especially late S phase) are most resistant
- Cells in very late G2 and M are most sensitive
- Variations probably due to ability of cells to repair damage by homologous recombination
- Sensitivity:

\[ M > G2 > G1 > S \text{ early} > S \text{ late} \]
Redistribution

• Effect of on a group of cells which are at various points in the cell cycle is to make the population that survive irradiation more synchronous
  • e.g. more cells in S phase have maintained their reproductive integrity
• During a course of fractionated RT, proliferating cells will move from 1 phase to another between doses
• 2 effects can make the cell population more sensitive to subsequent radiation
  • 1) Some of cells will be blocked in G2 phase of cycle (mitotic delay) which is a sensitive phase of the cycle
  • 2) Some of surviving cells will redistribute into more sensitive parts of the cycle

Redistribution makes the cell population more sensitive to fractionated treatment as compared with a single dose
Reoxygenation

• Process by which hypoxic clonogenic cells become better oxygenated during period after irradiation of a tumour
• Tumours <1mm fully oxygenated
• Above this size they usually become partially hypoxic
• Supply of oxygen affects the potency of RT
  • thought to be caused by the generation of ROS (reactive oxygen species)
• Oxygen assists in making radiation-induced damage permanent
• More double-strand breaks occur in cells irradiated in the presence of than in the absence of oxygen
Reoxygenation

• If tumours are irradiated with a large single dose of RT: most of radiosensitive aerobic cells will be killed
• Cells that survive will predominantly be hypoxic
  ➢ radiobiological hypoxic fraction immediately after RT will be close to 100%
• Subsequently hypoxic fraction falls due to reoxygenation and approaches its initial starting value

Reoxygenation can result in a substantial increase in sensitivity of tumours during fractionated RT
  • Probably major reason why fractionating treatment leads to an improvement in therapeutic ratio as compared to single large doses
Cell survival curve illustrating Rexoxygenation

- Wouters and Brown 1997
Radiosensitivity

• Cellular radiosensitivity
  • Sensitivity of cells to ionizing radiation in vitro
• Tissue radiosensitivity
  • Tissue vary in radiosensitivity due to functional organisation, level of proliferation and ability to undergo apoptosis
• Individual radiosensitivity
  • Intrinsic radiosensitivity is that which is genetically determined
  • Actual radiosensitivity is influenced by cell/tissue type and assay (cellular/tissue) and lifestyle factors (individual)
Measuring radiosensitivity

- Cellular radiosensitivity usually measured using a radiation survival curve using parameters such as
  - surviving fraction at low doses (e.g. SF2)
  - Dbar (area under a radiation survival curve)
- Gold standard for measuring radiosensitivity is a clonogenic assay
  - Single cells plated and colonies counted after 1-4 weeks
- A variety of assays have been/are studied to measure tumour radiosensitivity
  - Many assays not sufficiently reliable to be used in clinical studies
Differences in radiosensitivity reflect response to RT

- Variable susceptibility to cell death
  - contributes to radiosensitivity
  - may partly explain clinical heterogeneity within and between cancer types

- Interest in investigating new technologies for measuring multiple genes and deriving molecular profiles/signature

<table>
<thead>
<tr>
<th>Cell lines derived from</th>
<th>SF2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastoma, lymphoma, myeloma</td>
<td>0.19</td>
</tr>
<tr>
<td>Medullablastoma, small-cell lung ca.</td>
<td>0.22</td>
</tr>
<tr>
<td>Breast, bladder, cervix ca.</td>
<td>0.46</td>
</tr>
<tr>
<td>Pancreas, colorectal, squamous lung ca.</td>
<td>0.43</td>
</tr>
<tr>
<td>Melanoma, osteosarcoma, glioblastoma, renal ca.</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Deacon et al. 1984
<table>
<thead>
<tr>
<th></th>
<th>Total dose required for a given level of damage</th>
<th>Tumours</th>
<th>Early-responding normal tissues</th>
<th>Late-responding normal tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repair</td>
<td>![Up Arrow]</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Repopulation</td>
<td>![Up Arrow]</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Redistribution</td>
<td>![Down Arrow]</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Reoxygenation</td>
<td>![Down Arrow]</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Radiosensitivity</td>
<td>![Down Arrow]</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Further reading: Potential relationships between the 5Rs of radiobiology and the hallmarks of cancer

J.S. Good, K. Harrington, Clinical Oncology Volume 25, p569, 2013
Cell survival and the alpha-beta ratio

The linear quadratic model

- Cell survival curve can be described by equation:
  \[-\ln (\text{Survival}) = \alpha D + \beta D^2\]

- \(\alpha/\beta\) describes bendiness of the cell-survival curve
  - Often used to quantify fractionation sensitivity of tissues

- Initially established that \(\alpha/\beta\) was
  - High: for most tumours and acutely responding tissues (>8 Gy)
  - Low: for late-responding normal tissues (3-4 Gy)
The alpha-beta ratio

- Late responding tissues have lower $\alpha/\beta$ ratio and so curve is more bendy

- Late tissues are more sensitive to change in fraction size

- LQ model used to calculate effectiveness of various RT dose schedules
  - E.g. 20 Gy in 5#
  - E.g. calculate equivalent schedule in 2 Gy fractions which would give same biological effect (EQD$_2$)
  - Use different values of $\alpha/\beta$ to study acute and late effects
The alpha-beta ratio: New data

• New evidence suggests that $\alpha/\beta$ for some tumours is lower than that of surrounding tissues
  • Clinical and pre-clinical data suggest that prostate cancer has a low $\alpha/\beta$ ratio of 1.4-1.9 Gy
    ➢ tumour cells should be more sensitive to increased dose / fraction
  • 5 year efficacy and toxicity outcomes from 4 phase III trials (n>6000) have been published since 2016
    • Moderate hypofractionation (3.0-3.4 Gy / fraction) non-inferior to conventional fractionation (1.8-2.0 Gy per fraction)
    • Consistent with $\alpha/\beta$ of 1.3 -1.8 Gy

Reviewed in The Role of Hypofractionated Radiotherapy in Prostate Cancer, Benjamin, Tree, Dearnaley; Curr Oncol Rep (2017)
But it is a bit more complicated......

• Also depends on the ‘volume effect’....whether an organ at risk exhibits serial or parallel behaviour

• Serial: disabling a single functional subunit disables the whole organ, e.g. spinal cord

• Parallel: functional subunits arranged in parallel so organ can still function even if several functional subunits disabled, e.g. lung

The Radiobiology of Hypofractionation, A. Nahim, Clin Onc 27 (2016)
Normal tissue damage

- Acute effects
  - Increased with shorter treatment time due to lower repopulation of early responding tissues
  - $\alpha/\beta$ range 7-20 Gy
  - Less sensitive to changes in dose/fraction

- Late effects
  - Less dependent on overall treatment time
  - $\alpha/\beta$ range 0.5-6 Gy
  - More sensitive to changes in dose/fraction

$\alpha/\beta = \text{Ratio of the parameters } \alpha \text{ and } \beta \text{ in the linear-quadratic model}$
QUANTEC

• Quantitative Analysis of Normal Tissue Effects in the Clinic
• Int J Radiat Oncol Biol Phys 76 (3 Supplement) 2010 (open access)
• QUANTEC reviews in 2010 provide focused summaries of the dose/volume/outcome information for 16 organs at risk
  • E.g. brain, spinal cord, parotid gland, lung, kidney, bowel etc.
# QUANTEC

## Table 1. QUANTEC Summary: Approximate Dose/Volume/Outcome Data for Several Organs Following Conventional Fractionation (Unless Otherwise Noted)*

<table>
<thead>
<tr>
<th>Organ</th>
<th>Volume segmented</th>
<th>Irradiation type (partial organ unless otherwise stated)</th>
<th>Endpoint</th>
<th>Dose (Gy) for dose/volume parameters</th>
<th>Rate (%)</th>
<th>Notes on dose/volume parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain</strong></td>
<td>Whole organ</td>
<td>3D-CRT</td>
<td>Symptomatic necrosis</td>
<td>(D_{\text{max}} = 60)</td>
<td>&lt;3</td>
<td>Data at 72 and 90 Gy, extrapolated from BED models</td>
</tr>
<tr>
<td></td>
<td>Whole organ</td>
<td>3D-CRT</td>
<td>Symptomatic necrosis</td>
<td>(D_{\text{max}} = 72)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole organ</td>
<td>3D-CRT</td>
<td>Symptomatic necrosis</td>
<td>(D_{\text{max}} = 90)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole organ</td>
<td>SRS (single fraction)</td>
<td>Symptomatic necrosis</td>
<td>(V_{12} &lt; 5–10 \text{ cc})</td>
<td>&lt;20</td>
<td>Rapid rise when (V_{12} &gt; 5–10 \text{ cc})</td>
</tr>
<tr>
<td><strong>Brain stem</strong></td>
<td>Whole organ</td>
<td>Whole organ</td>
<td>Permanent cranial neuropathy or necrosis</td>
<td>(D_{\text{max}} &lt; 54)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole organ</td>
<td>3D-CRT</td>
<td>Permanent cranial neuropathy or necrosis</td>
<td>(D_{1–10 \text{ cc}} &lt; 59)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole organ</td>
<td>SRS (single fraction)</td>
<td>Permanent cranial neuropathy or necrosis</td>
<td>(D_{\text{max}} &lt; 64)</td>
<td>5</td>
<td>Point dose &lt;&lt; 1 cc</td>
</tr>
<tr>
<td><strong>Optic nerve / chiasm</strong></td>
<td>Whole organ</td>
<td>3D-CRT</td>
<td>Optic neuropathy</td>
<td>(D_{\text{max}} &lt; 55)</td>
<td>3-7</td>
<td>Given the small size, 3D-CRT is often whole organ(^{13})</td>
</tr>
<tr>
<td></td>
<td>Whole organ</td>
<td>3D-CRT</td>
<td>Optic neuropathy</td>
<td>(D_{\text{max}} &lt; 60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole organ</td>
<td>SRS (single fraction)</td>
<td>Optic neuropathy</td>
<td>(D_{\text{max}} &lt; 12)</td>
<td>10</td>
<td>For patients with acoustic tumors</td>
</tr>
<tr>
<td><strong>Spinal cord</strong></td>
<td>Partial organ</td>
<td>3D-CRT</td>
<td>Myelopathy</td>
<td>(D_{\text{max}} = 50)</td>
<td>0.2</td>
<td>Including full cord cross-section</td>
</tr>
<tr>
<td></td>
<td>Partial organ</td>
<td>SRS (single fraction)</td>
<td>Myelopathy</td>
<td>(D_{\text{max}} = 13)</td>
<td>1</td>
<td>Partial cord cross-section irradiated</td>
</tr>
<tr>
<td></td>
<td>Partial organ</td>
<td>SRS (hypofraction)</td>
<td>Myelopathy</td>
<td>(D_{\text{max}} = 20)</td>
<td></td>
<td>3 fractions, partial cord cross-section irradiated</td>
</tr>
</tbody>
</table>
| **Cochlea**            | Whole organ      | 3D-CRT                                                   | Sensory neural hearing loss      | Mean dose \(\leq 45\)              | <30      | Mean dose to cochlear, hearing at 4

*Note: * Use of RTCP models in the clinic.
## QUANTEC

<table>
<thead>
<tr>
<th>Limitations</th>
<th>Aims</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficulty synthesizing results from different publications</td>
<td>To assist in determining acceptable dose constraints</td>
</tr>
<tr>
<td>Incomplete reporting results in studies</td>
<td>Provide recommendations on reporting and gathering data on dose-volume dependencies of treatment outcome</td>
</tr>
<tr>
<td>Use of incompatible or ambiguous endpoints</td>
<td>Provide recommendations for appropriate toxicity endpoints to use</td>
</tr>
<tr>
<td></td>
<td>To suggest areas for future research</td>
</tr>
<tr>
<td></td>
<td>Adoption of these recommendations will facilitate future meta-analysis and therefore increase use of individual studies</td>
</tr>
</tbody>
</table>
Further Reading

Thank you for your attention