Imaging Enhancement of Models of Disseminated Multiple Myeloma for Drug Discovery and MR Imaging

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Background
Multiple myeloma is a cancer of the plasma cells that is characterized by multiple localized lesions in the marrow, particularly of the spine, skull, and pelvis, although soft tissue lesions also occur. It is the second most common blood cancer, affecting approximately 45,000 people in the US. Most preclinical modeling of myeloma employs SC xenografts that mimic the less common plasmacytoma form of the disease. Systemic (IV) implants are also used, but studies are typically limited to a single survival endpoint, limiting knowledge about the progression and response of the disease under treatment. In order to more quantitatively monitor disseminated disease progression and response to treatment, we have characterized two human (JJN3 and MM1S) and one murine (STGMI) myeloma models that have been modified to express luciferase. In the described studies we show sensitivity to standards of care in these systemic systems as measured with bioluminescence imaging (BLI).

Methods

**JJN3 (pMMP-LucNeo) & MM1S (pMMP-LucNeo):**
- Female SCID-Beige mice were implanted intravenously with 5 x 10⁶ cells in 200µl of PBS.
- Growth media: RPMI + 10% FBS + 1% PSG + 1% L-Glut

**STGMI-luc:**
- Female NIH III (bg/nd/xid) mice were implanted intravenously with 5 x 10⁶ cells in 200µl of DPBS.
- Growth media: RPMI + 15% FBS + 1% PSG

**Imaging and Analysis**
- BLI occurred using an IVIS 50 optical imaging system (Xenogen, Alameda, CA) at multiple time points throughout the lifespan of the animals.
- Luciferin (150mg/kg) dosed IP and animals were anesthetized using isoflurane anesthesia.
- Each animal imaged 10 minutes post luciferin injection.
- In vivo whole body tumor burden and focal disease (in vivo and ex vivo) were quantified via bioluminescent light emission in photons/sec.

**Results**
All models were characterized by 100% tumor take rate and focal dissemination of the disease to the spine and skull that mimic clinical experience. These models showed individual and reproducible patterns of spread to other sites, and differed in their sensitivities to standards of care. Analysis of tumor doubling times, tumor titrations, and survival all indicated that the bioluminescence signal was a reliable quantitative indicator of viable tumor burden, and response to treatment with clinical standard of care agents. The kinetics of the BLI signal was directly related to tumor progression and overall survival times, and allowed the measurement of a true tumor growth delay, and tumor burden regressions without waiting for survival endpoints.

**Conclusion:**
Noninvasive imaging provides the only efficient way of quantifying the real time progression of disseminated cancers and their response to treatment. This methodology also reduces the use of animals and drug candidates necessary for a complete efficacy study. The use of BLI derived tumor growth delays and subsequent calculation of surviving fractions, delivers truly quantitative endpoints far sooner than can be achieved with survival endpoints, speeding the discovery process.

**Incidence of Engraftment**

<table>
<thead>
<tr>
<th>Tumor Line</th>
<th>Hind Limbs</th>
<th>Spine</th>
<th>Lungs</th>
<th>Mandible</th>
<th>Brain</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>JJN3 (pMMP-LucNeo)</td>
<td>76%</td>
<td>95%</td>
<td>92%</td>
<td>92%</td>
<td>38%</td>
<td>17%</td>
</tr>
<tr>
<td>MM1S (pMMP-LucNeo)</td>
<td>100%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>100%</td>
</tr>
<tr>
<td>STGMI-luc</td>
<td>100%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>100%</td>
</tr>
</tbody>
</table>

* P<0.05 vs. Control
** Based off of BLI signal