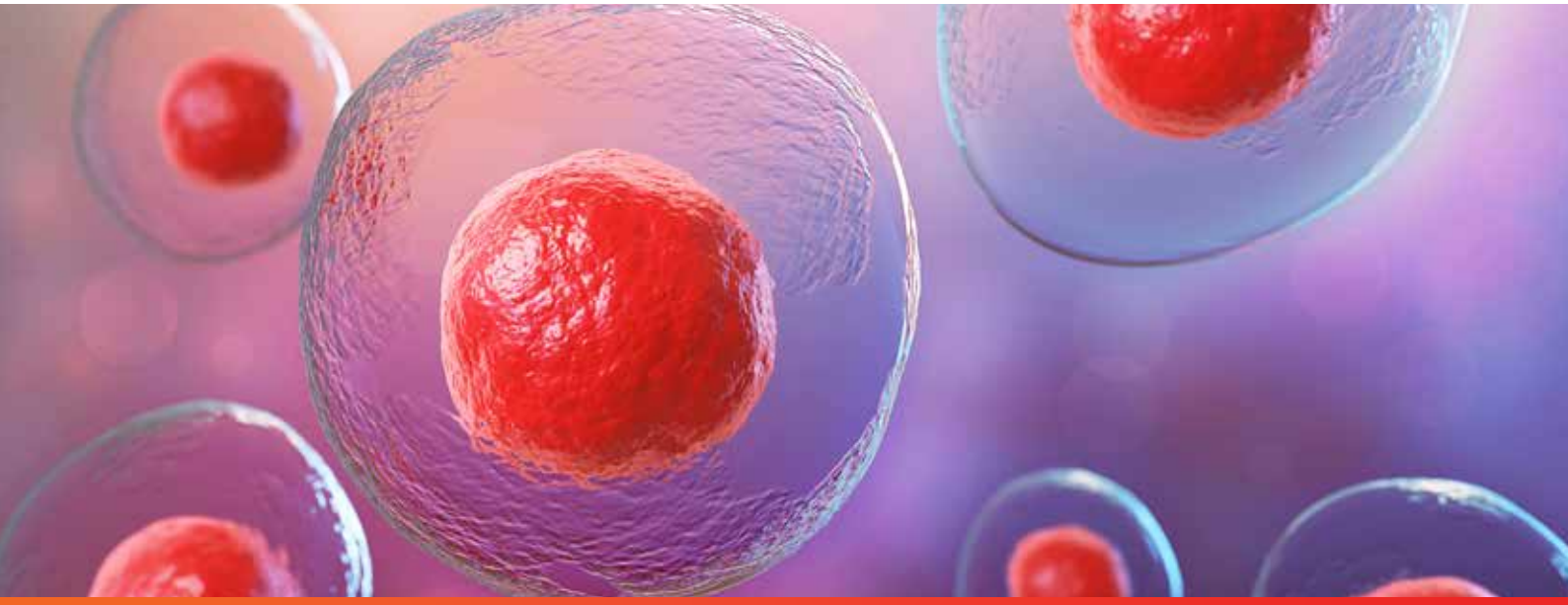
aragen.com



Aragen's Preclinical Oncology Capabilities - Tumor Imaging

Executive Summary

Imaging is a critical tool for non-invasive assessment of biological and biochemical processes in living subjects. IND-enabling studies of any new therapeutics is incomplete without relevant images capturing targeted biological and biochemical processes. Imaging tools help us decide or select drug candidates that most likely succeed in a vigorous clinical trial process. Here, we present few preclinical oncology case studies, for example breast and ovarian cancer models that show key role of molecular imaging in drug development process.

Introduction

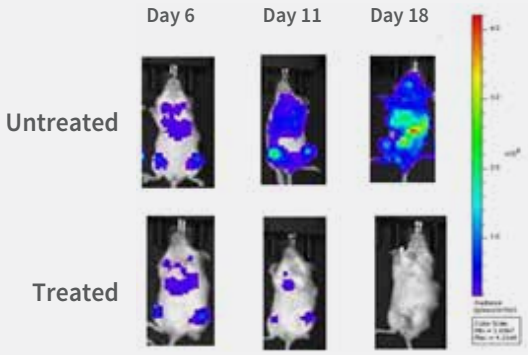
Non-invasive imaging plays a critical role in preclinical and clinical anticancer drug development process. Imaging is part of most protocols for conducting preclinical and clinical studies of various anticancer drugs since it provides morphological, structural, metabolic, and functional information [1] of drug-target interactions. In clinics, biomedical imaging serves as the foundation of comprehensive cancer care, offering benefits such as non-invasive real-time monitoring of biological and pathological processes [1]. Additionally, biomedical imaging plays a crucial role in cancer management, including prediction [2], screening [3], biopsy guidance for detection [4], staging [5], prognosis [6], and therapeutic guidance [7]. More importantly, imaging-based screening for early diagnosis continues to play a most important factor in lowering cancer mortality [8].

Aragen's competence as a leader in preclinical oncology services is built on more than a decade of anticancer drug development using appropriate animal models and precision molecular imaging tools. Aragen scientific team will design, customize, and execute the preclinical oncology studies with major input form the clients and partners. Our entire teamwork in tandem with the clients to ensure and expedite workflow in a cost-effective and timely manner. Our state-of- the-art imaging facilities include Multi species optical and X-ray imaging, advanced *in-vivo* fluorescent and bioluminescent imaging and Multiplex Immunophenotyping. This article will show case few preclinical oncology case studies using Syngeneic, Xenografts, Orthotopic and metastatic tumor models. The following in-house studies highlight the key role of molecular imaging in the drug development process.

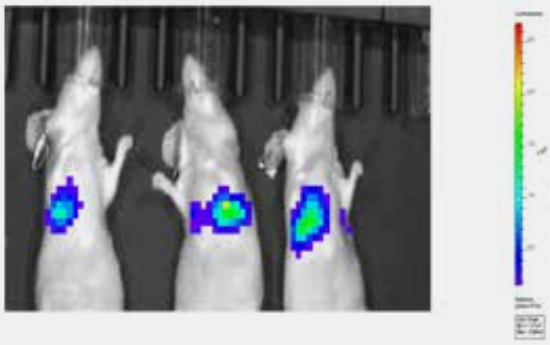
Case Study 1: Multi species optical and X-ray Imaging

PE IVIS XRMS System for multi-species optical and X-ray Imaging was used to perform live imaging of tumours developed in Raji-Luc Disseminated model implanted with metastatic A549-LuC cells. The Luciferase signals captured intensified over 6-18 days of tumour implantation, whereas the signals were weak in treated mice. Similarly, luciferase signal was captured in lungs in intraperitoneal or sub-cutaneous tumours in SKOV-3 xenograft model and IntegriSenseTM 645 signal in BX PC3-Luc orthotopic model.

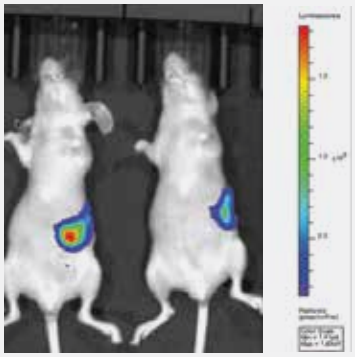
Live Imaging of Raji-Luc Disseminated Model



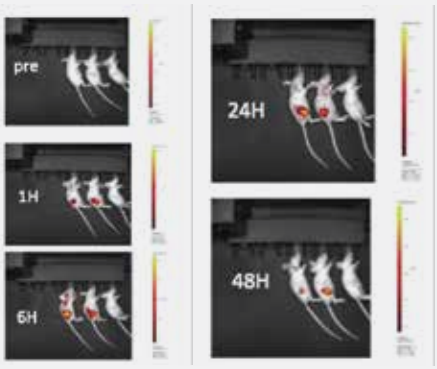
Live Imaging of A549-Luc Lung Metastasis Model



Living Imaging of BX PC3-luc Orthotopic Model

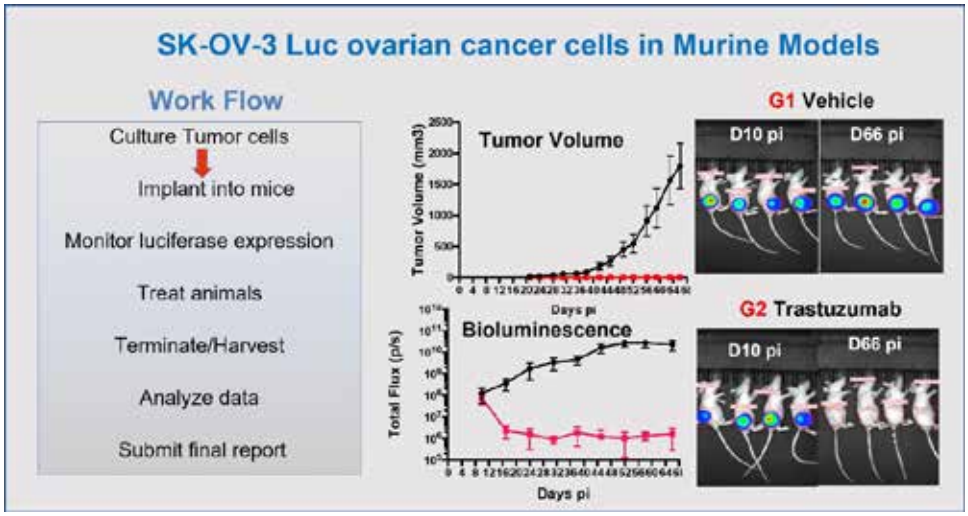


Live Imaging of SKOV-3 xenograft with IntegriSense™ 645



Case study 2: *In-Vivo* Bioluminescent Imaging

This study in murine tumor shows the early detection of SK-OV-3 Luc ovarian cancer cells implanted sub-cutaneous regions before they form clearly visible tumors. Such a study might give a tumor development timeline that may be used to develop a window for treatment with anti-cancer therapeutics. The graphs show the increase in tumor volume over time and the treatment with Trastuzumab reduced the tumor growth. Luciferase imaging intensity was high in untreated mice while the intensity decreased in treated mice, suggesting the anti-tumor effect of Trastuzumab. Also shown is the workflow for this study developed in consultation with the clients or partners.

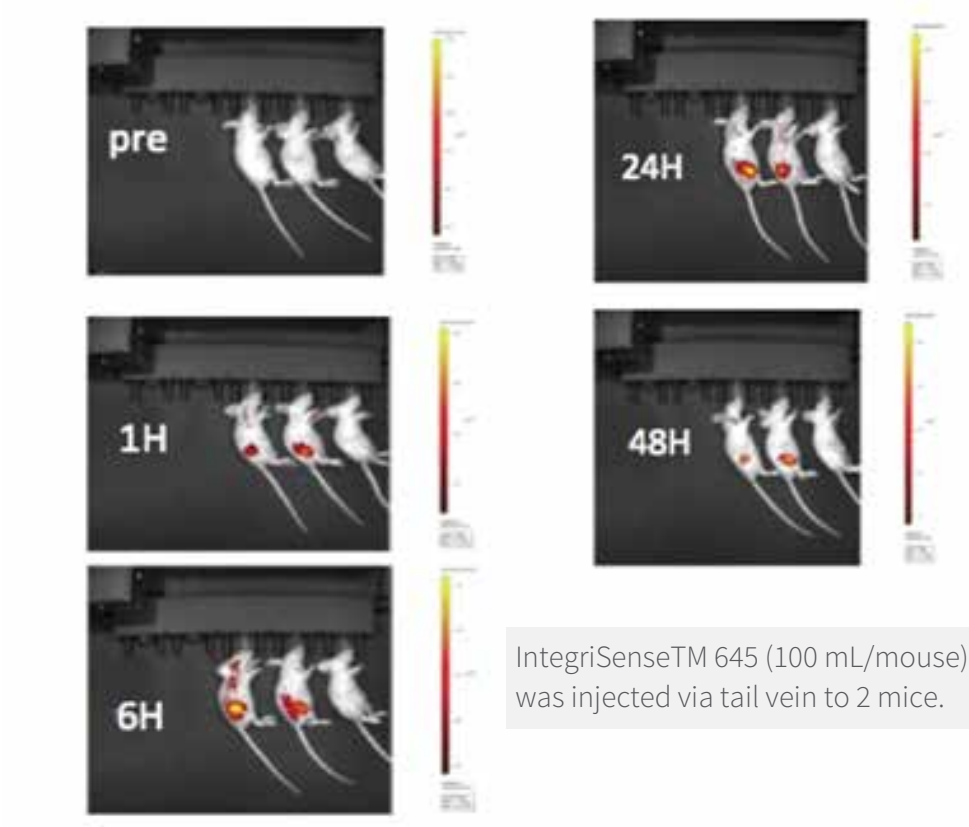


Case study 3: *In-Vivo* Fluorescent Imaging in SKOV-3 xenograft using IntegriSense TM 645

IntegriSense is a targeted fluorescent imaging agent comprising a potent, selective non-peptide small molecule integrin avb3 antagonist tagged with NIR fluorochrome. It was developed to enable in vivo visualization and quantification of integrin expressed in tumor cells as well as in neo vasculature, and monitor tumour growth, tumor angiogenesis and treatment efficacy.

This study in murine xenograft model shows the images of sub-cutaneous SK-OV-3 tumor at 1-48 hrs. post IntegriSense TM 645 administration (100 mL/mouse) via tail vein to 2 mice (LEFT) and vehicle administered to 1 mouse (RIGHT). Note that the tumor is undetectable in pre-IntegriSense TM 645 administered mice.

Living Imaging of SKOV-3 xenograft with IntegriSense ™ 645



IntegriSenseTM 645 (100 mL/mouse) was injected via tail vein to 2 mice.

Case study 4: Multiplex Immunophenotyping Capability

We performed Immunophenotyping in different call lines using Attune (14 colour FACS) as shown in the following table. Different subsets of immune cells were tagged, and different T cell counts were measured.

Methodology: Immunophenotyping couples' specific antibody to a fluorescent compound and helps in measuring specific protein expression within a cell population. The protein expressed in certain cells are used to identify and categorize the population of tagged cells. It is often used to measure CD4-T cell counts in specific immunodeficiency disorders and immune-related diseases. It is also used to detect the presence or absence of biomarkers of cancer in cancer cells to predict their severity in forming tumors and to study targeted drug effects.

Immune cell subset	Markers (Mouse) up to 14 colors
Effector CTL cell	$CD45^+$ $CD3^+$ $CD4^-$ CDS^+ $CD44$ $'^{-high}$
Regulatory T cell	$CD45^+$ $CD3^+$ $CD4^+$ $CD8^-$ $CD25^+$ $FoxP3^+$
Total MDSCs	$CD45^+$ $CD3^-$ $CD11^+$ $Gr1^+$
M-MDSCs	$CD45^+$ $CD3^-$ $CD11b^+$ $Gr1^+$ $Ly6G^-$ $Ly6C^+$
G-MDSCs	$CD45^+$ $CD3^-$ $CD11b^+$ Grl^+ Ly6G+ $Ly6C^-$
M1 Macrophage	$CD68^+$ $CD80^+$ $CD206^-$
M2 Macrophage	$CD68^+$ $CD80^-$ $CD206^+$
B cell	$CD45R/B220^+$
NK cell	$CD335^+$ $CD49b^+$
pan NK cells	$CD11b^{Low}$ $CD49b^+$ $(DX5)^+$
Adaptive Immunity (T cells, NK, NKT)	$CD45^+CD3^+$ $CD4^+$ $CD8^+$ TCR^+ Fox $P3^+$ yd-TCR^+ $PD\text{-}1^+$ $CD49b^+$ $DX5^+$ $CD69^+$

References:

1. Fass, L., 2008. Imaging and cancer: a review. Molecular oncology, 2(2), pp.115-152.
2. de Torres, J.P., Bastarrika, G., Wisnivesky, J.P., Alcaide, A.B., Campo, A., Seijo, L.M., Pueyo, J.C., Villanue va, A., Lozano, M.D., Montes, U. and Montuenga, L., 2007. Assessing the relationship between lung cancer risk and emphysema detected on low-dose CT of the chest. Chest, 132(6), pp.1932-1938.
3. Lehman, C.D., Isaacs, C., Schnall, M.D., Pisano, E.D., Ascher, S.M., Weatherall, P.T., Bluemke, D.A., Bowen, D.J., Marcom, P.K., Armstrong, D.K. and Domchek, S.M., 2007. Cancer yield of mammography, MR, and US in high-risk women: prospective multi-institution breast cancer screening study. Radiology, 244(2), pp.381-388.
4. Nelson, E.D., Slotoroff, C.B., Gomella, L.G. and Halpern, E.J., 2007. Targeted biopsy of the prostate: the impact of color Doppler imaging and elastography on prostate cancer detection and Gleason score. Urology, 70(6), pp.1136-1140.
5. Kent, M.S., Port, J.L. and Altorki, N.K., 2004. Current state of imaging for lung cancer staging. Thoracic Surgery Clinics, 14(1), pp.1-13.
6. Lee, K.S., Jeong, Y.J., Han, J., Kim, B.T., Kim, H. and Kwon, O.J., 2004. T1 non–small cell lung cancer: imaging and histopathologic findings and their prognostic implications. Radiographics, 24(6), pp.1617-1636.
7. Ciernik, I.F., Dizendorf, E., Baumert, B.G., Reiner, B., Burger, C., Davis, J.B., Lütolf, U.M., Steinert, H.C. and Von Schulthess, G.K., 2003. Radiation treatment planning with an integrated positron emission and computer tomography (PET/CT): a feasibility study. International Journal of Radiation Oncology* Biology* Physics, 57(3), pp.853-863.
8. Ashamalla, H., Rafla, S., Parikh, K., Mokhtar, B., Goswami, G., Kambam, S., Abdel-Dayem, H., Guirguis, A., Ross, P. and Evola, A., 2005. The contribution of integrated PET/CT to the evolving definition of treatment volumes in radiation treatment planning in lung cancer. International Journal of Radiation Oncology* Biology* Physics, 63(4), pp.1016-1023.