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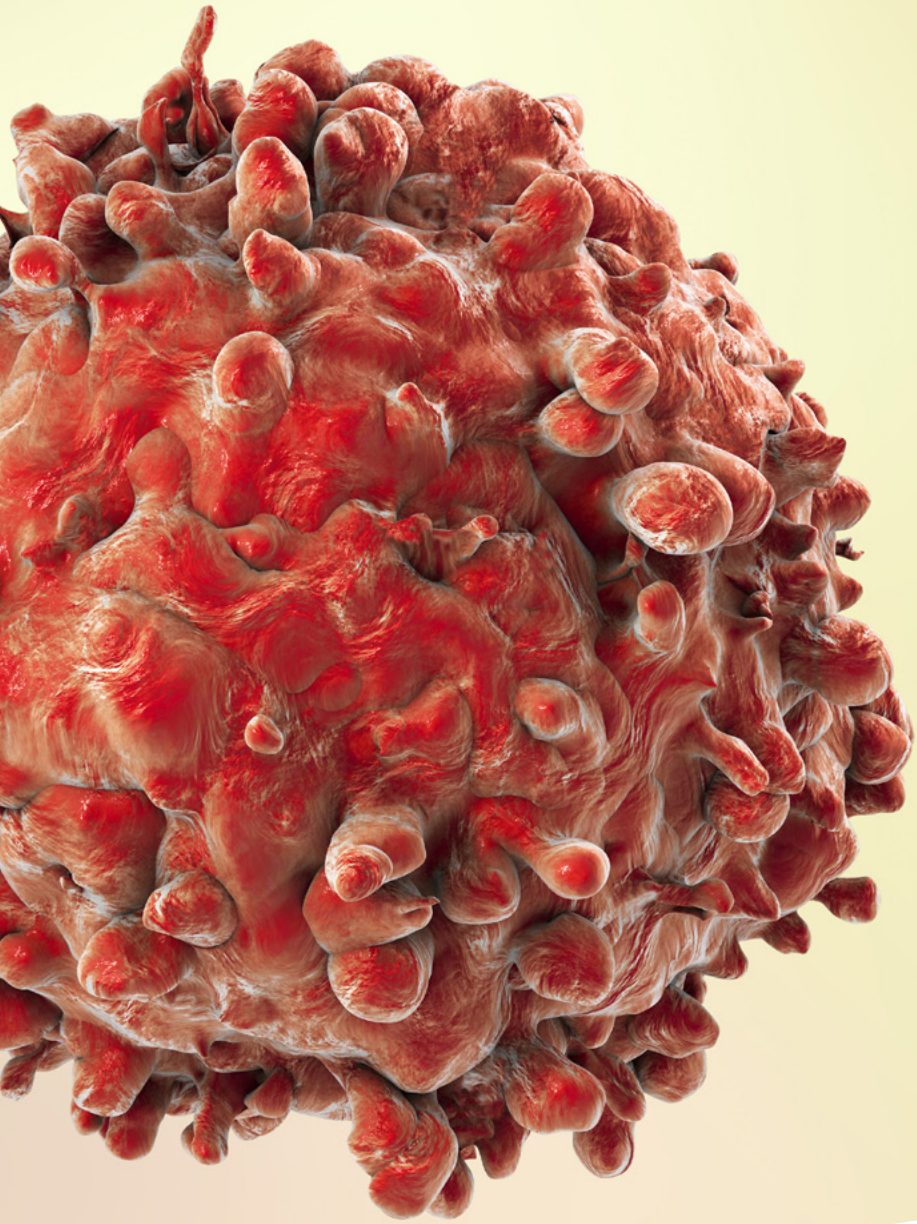


White Paper // April 2019

PDX, CDX and Allograft Growth

IN THE R2G2® MOUSE

Contents

Introduction

An important step in the preclinical testing of investigational cancer therapies is the use of immunodeficient mice as hosts for human tumor xenografts arising from cell lines or tumor tissue (see definitions in box). These immunodeficient mice have been an invaluable resource for cancer researchers (among other research fields) and they have greatly facilitated the evaluation and advancement of countless investigational cancer therapies, and their utility continues to expand as they are being used for an increasing number of oncology-related applications, including discovering biomarkers, testing precision-medicine approaches, developing therapies for various tumor stages (e.g., early versus advanced), and treating drug-resistant tumors.

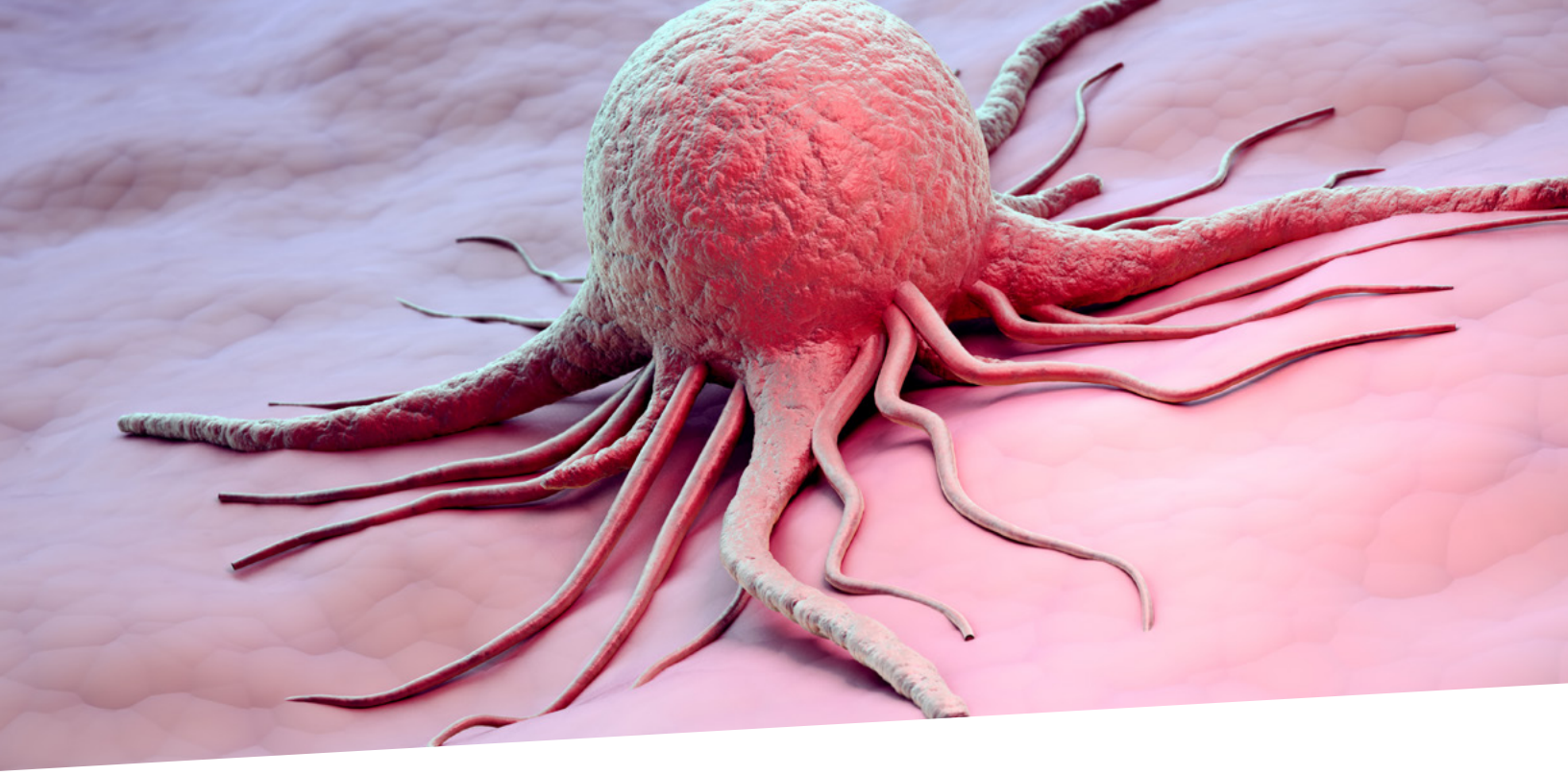
The development of patient-derived xenograft (PDX) models has been especially invaluable for oncology research since these models have been shown to carry the original tumor characteristics, such as heterogeneity, complexity, and molecular diversity (Xu 2019). PDX models have been successfully established using a range of tumor types, including (among others) breast, lung, colorectal, melanoma, and ovarian tumors (Xu 2019; Pompili 2016), and PDXs have been used for a variety of purposes, including drug development (i.e., good predictive utility for clinical outcomes) (Gao 2015) and PDX-guided therapy where models are used to help select optimal treatment strategies for patients (Xu 2019).

Definitions of terms used in this paper

Patient-Derived Xenograft (PDX):
Immunodeficient animals are engrafted with human-derived tumor tissue.

Cell Line Xenograft (CDX):
Immunodeficient animals are engrafted with human tumor cell lines.

Allograft:
Host animals are engrafted with tumor tissue or tumor cell lines derived from the same genetic background as the animal.



In contrast to PDX models, CDX models are generated by engrafting established human cell lines into immunodeficient mice, and these have also been shown to have predictive value (Jung 2014; Johnson 2001; Langdon 1994). Together, PDX and CDX models are important tools that will continue to be part of the expanding armamentarium available to cancer researchers that is enabling amazing breakthroughs in our understanding of cancer biology and enabling the development of novel anticancer therapies.

The B6;129-*Rag2*^{tm1Fwa}*IL2rg*^{tm1Rsky}/DwlHsd (R2G2®) knockout mouse is an ultra-immunodeficient model that is commercially available from Inotiv. Since new studies using the R2G2® strain are just beginning to emerge in the literature (Page 2019; Hong 2018; Jimenez-Segovia 2018) [see Table 1 for a summary of these studies], the available publications on this strain are currently limited.

This paper presents a compilation of tumor growth data from several PDXs, CDXs, and allografts in the R2G2® mouse. Specifically, data are from two head and neck cancers for PDXs, esophageal (OE33 and FLO1) and gastric (AGS) cancer for CDXs, and murine colorectal cancer (CT26) and B-cell lymphoma (A20) cells for allografts. The data presented in this paper can assist researchers in choosing the optimal immunodeficient strain for their cancer research programs.

Background information on the R2G2® mouse is provided first, including its immunodeficient characteristics and its tolerance of some standard chemotherapeutic agents and estrogen supplementation.

Recent studies using the R2G2® Mouse

HONG ET AL. STUDY	
Objective:	To evaluate the effects of targeting the AXL receptor tyrosine kinase in a CDX model of esophageal adenocarcinoma.
Methods:	R2G2® mice were used to generate a CDX model using FLO1 cells injected into the flanks. Once tumors reached a volume of 500 mm^3 (after approximately 30 days) treatments were initiated for a period of 10 days using epirubicin, R428 (an inhibitor of AXL), or a combination of these two agents.
Results:	There was a synergistic suppression of tumor growth and proliferation when the animals were treated with epirubicin plus R428 compared to either agent alone. Investigators concluded that these data support future clinical trials to test the therapeutic potential of R428 and epirubicin in tumors that overexpress AXL *(Hong 2018)*.

JIMENEZ-SEGOVIA ET AL. STUDY	
Objective:	To assess the role of *PMEPA1* in cancer progression, specifically in for ovarian cancer.
Methods:	Several CDX models, including models developed using the R2G2® mouse, were generated using two human ovarian cancer cell lines: TOV112D and A2780 (control and *PMEPA1* overexpressing cells).
Results:	For TOV112D cells that overexpressed *PMEPA1*, the authors found that tumor initiation rates and growth rates were enhanced as compared to controls. For the A2780 experiment, tumors did not form in R2G2® mice injected with control cells, but the *PMEPA1* overexpressing cells developed tumors *(Jimenez-Segovia 2018)*.

PAGE ET AL. STUDY	
Objective:	To elucidate the role of IKKα in lung cancer.
Methods:	H460 lung cancer cell lines expressing exogenous IKKα either in the nucleus (N-IKK) or in the cytoplasm (C-IKK) were generated. R2G2® and athymic nude mice were injected with the three cell lines (control, N-IKK, and C-IKK) to develop the CDX models.
Results:	In R2G2® and athymic nude mice, the latency period of the tumors was 9 days for the C-IKK and N-IKK cells, compared to 11 days in the control cells. At 11 days, tumors from the C-IKK and N-IKK cells were larger than control tumors, and this difference was maintained at subsequent time points. Overall, C-IKK and N-IKK were shown to enhance tumor growth and progression compared to controls *(Page 2019)*.

The R2G2® model

The R2G2® mouse is genetically engineered and harbors mutations in the recombination-activating gene 2 (*Rag2*) (Mombaerts 1992; Shinkai 1992) and the *IL2rg* (common gamma chain) gene product (Puck 1997; Mazurier 1999; Goldman 1998). In 2016, Inotiv acquired the R2G2® mouse from the Fox Chase Cancer Center (Philadelphia, PA), where it had been maintained since 2005. It was generated by backcrossing an *IL2rg*-mutated mouse on a mixed background (C57BL/6 and 129) with a mouse bearing the *Rag2* mutation. The result was a double-knockout mouse with an ultra-immunodeficient phenotype. The combination of the *Rag2* and *IL2rg* mutations in the R2G2® mouse provides a model that lacks various cytokines, including IL-2, IL-4, IL-7, IL-9, and IL-15. The R2G2® also lacks B cells, T cells, and Natural Killer (NK) cells and has a deficit in dendritic cells, macrophages, and lymphocyte development.

The R2G2® mouse has also been shown to have a reduced radiosensitivity compared to the NOD *scid* gamma mouse (brand name NSG™), which is known to be hypersensitive to ionizing radiation (see the Inotiv paper, "A comparative analysis of R2G2® and NSG™ radiosensitivity"). The decreased radiosensitivity of the R2G2® mouse has positioned it to be advantageous for studies requiring radiation, such as radiation-therapy studies.

Other limitations of SCID mice include enhanced susceptibility to adverse effects of standard-of-care chemotherapy drugs and estrogen (also commonly observed in athymic nude mice). Evaluating investigational cancer agents often requires comparative head-to-head testing against or combination with standard-of-care chemotherapeutic drugs. Many human tumor xenograft models also require treatment with estrogen for optimal tumor growth. Highlighted below are data showing the significant tolerance of the R2G2® mouse for standard-of-care chemotherapy drugs and estrogen treatment.

Tolerance for chemotherapeutics and estrogen supplementation

The tolerance of Inotiv's R2G2® mouse for several standard-of-care chemotherapy drugs and doses of estrogen supplementation has been tested with respect to overall survival rate and body weight. For researchers already committed to using the R2G2® mouse, these data can inform about the best doses. For scientists not using the R2G2® mouse, these data provide key insights to determine whether it might be an optimal choice for the preclinical testing of their investigational cancer agents. The following sections provide an abridged version of the data presented in the Inotiv white paper (April 2018) titled, *"Tolerability of R2G2® Mouse to Chemotherapy and Estrogen Supplementation".*

CHEMOTHERAPY DRUGS

To evaluate tolerance for standard-of-care chemotherapy drugs, scientists treated R2G2® mice with typical experimental levels of 5-fluorouracil (5-FU), doxorubicin (Dox), or cyclophosphamide (CTX) and included an untreated control group. Dosing was up to three (Dox and CTX) or five (5-FU) weeks, and survival and body weight was followed for ~8 weeks (60 days). In addition to evaluating survival, blood samples were taken for hematology and clinical chemistry analysis as the mice became moribund or at the end of the study (in necropsy).

Regarding overall survival, all three standard-of-care chemotherapy drugs were well tolerated in the R2G2® mouse at the lower doses. For both 5-FU and Dox, there appeared to be levels above which toxicity increased sharply, as indicated by decreased survival. CTX was well tolerated at all doses.

Body weight data were consistent with the results seen in overall survival for the three chemotherapy drugs. The results from Dox treatment showed lower body weights at the 5 mg/kg dose versus the 2 mg/kg dose. At the lower dose of 5-FU (30 mg/kg), body weights were maintained, despite the degree of morbidity and weight loss seen at the higher doses. Tolerance of CTX was good at both doses (100 and 140 mg/kg), and body weights continued to increase at both doses over the course of the study (body weights were slightly below those of the control animals).

ESTROGEN SUPPLEMENTATION

To evaluate the tolerability of estrogen relative to overall survival and body weight for 60 days, estrogen pellets were implanted into R2G2® mice at doses up to 1.7 mg.

Specifically, R2G2® mice received a single 60-day release 17β-estradiol pellet (Innovative Research of America) at 0.18 mg, 0.36 mg, 0.72 mg, and 1.7 mg (n = 10/group). Body weight was monitored at regular intervals, and overall survival was monitored for up to 60 days.

Adverse effects for all doses were minor in terms of survival and body weight as compared to control animals. This lack of severe morbidity and stable weight over the study duration argue that these animals can serve as a consistent model for growth of estrogen-dependent tumors.

PDX, CDX, and allograft studies

Scientists evaluated tumor growth after implantation of human tumor tissue (PDX), human cell lines (CDX), and mouse cell lines (allograft) in R2G2® mice.

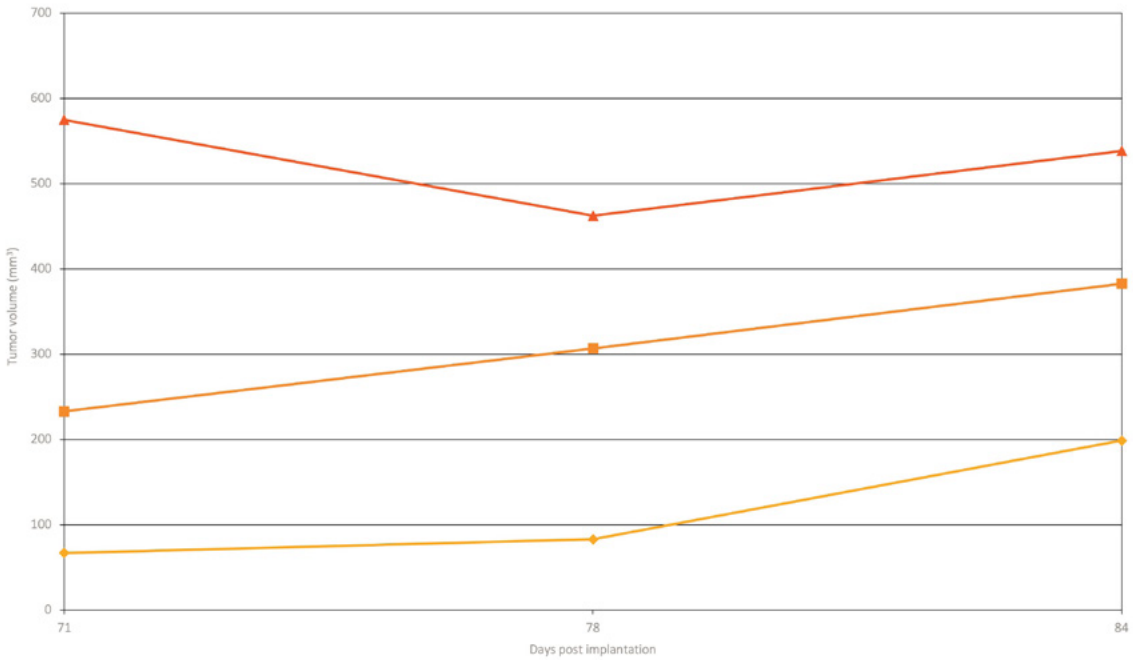
PDX models were generated by implanting tissue derived from two independent head and neck human tumors. Three CDX models were generated using cell lines originally derived from esophageal adenocarcinomas, gastric adenocarcinomas, and head and neck cancers (two different cell lines were used for each CDX model). For the esophageal and gastric adenocarcinoma studies, athymic nude and/or SCID mice were implanted for comparative purposes. Finally, two allograft studies were conducted using colon carcinoma and B-cell lymphoma cell lines.

The results of these studies are presented below.

PDX: HEAD AND NECK CANCER

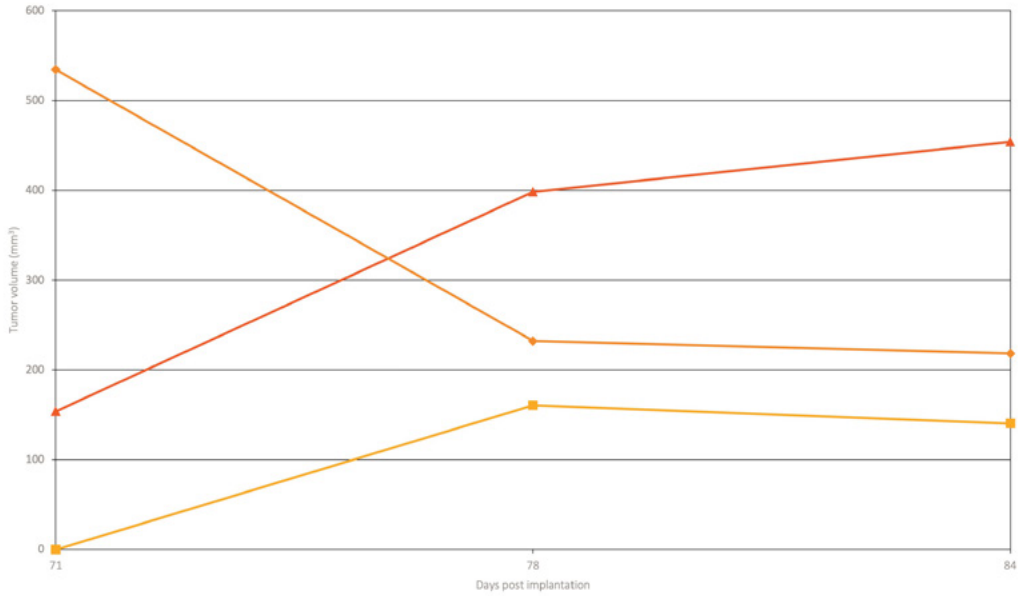
Two human head and neck tumor tissue samples (designated 626 and 635) were implanted into R2G2® mice to generate the PDX models. Each sample was sectioned into four pieces of approximately equal size (2.2 mm^2), and two pieces from each independent tumor were implanted into two R2G2® mice. Three 626 and three 635 tumors developed. The average tumor volume was 373.4 and 270.8 mm^3 on day 84 for PDX 626 and 635, respectively (Figures 1 and 2).

Figure 1: Human head and neck patient derived xenograft (PDX 626) tumor growth in R2G2 mice



Data are represented as individual tumors

Figure 2: Human head and neck patient derived xenograft (PDX 635) tumor growth in R2G2 mice



Data are represented as individual tumors

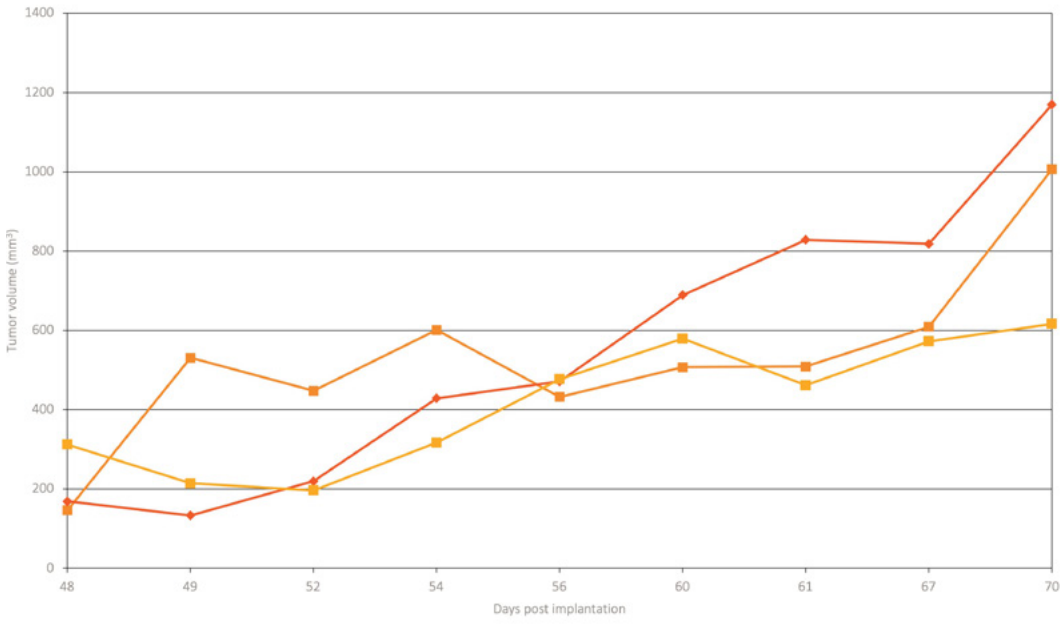
CDX: ESOPHAGEAL ADENOCARCINOMA

Two human esophageal adenocarcinoma cell lines (OE33 and FLO1) were implanted into R2G2® mice to generate the CDX models. Athymic nude and SCID mice were also implanted for comparison.

In the first study, OE33 cells (5x10^6 cells per mouse) were injected into the left and right flanks of SCID (n = 3), athymic nude (n = 3), and R2G2® (n = 2) mice.

The take rate of the OE33 cells was 100% in R2G2® mice, 17% in athymic nude mice, and 0% in SCID mice. Only one small tumor developed in the athymic nude mouse (data not shown). Average tumor growth on day 70 was 930.9 mm^3 in the R2G2® animals (Figure 3).

Figure 3: Human esophageal adenocarcinoma (OE33) tumor growth in R2G2 mice



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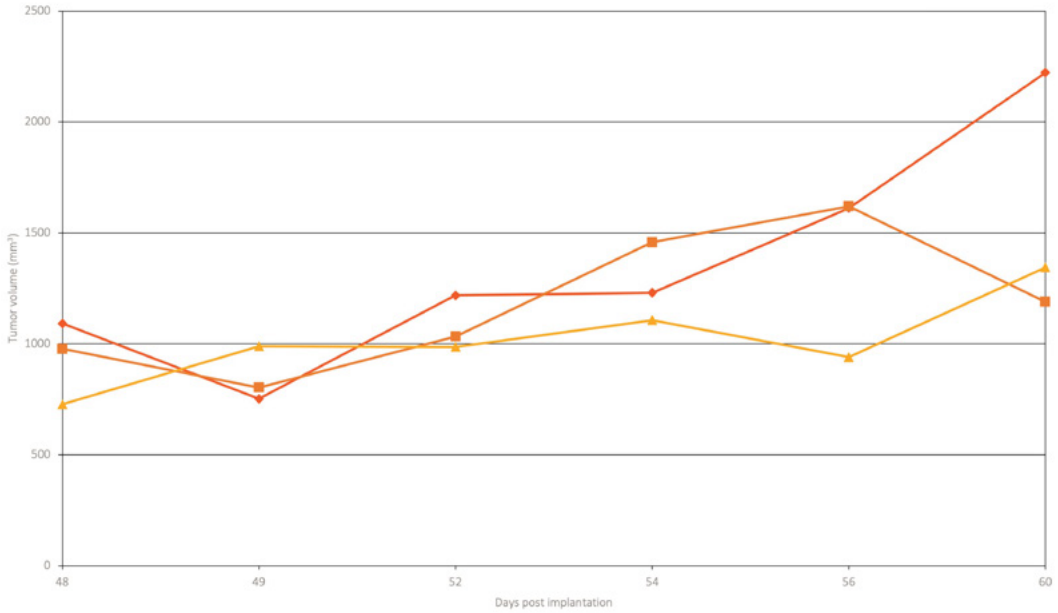
In the second study, FLO1 cells were evaluated in two sub-studies, designated Study A and Study B; twice as many cells were injected in Study B. In Study A, FLO1 cells ($5x10^6$ cells per mouse) were injected into both flanks of athymic nude (n = 3), SCID (n = 3), and R2G2® (n = 2) mice. In Study A, no tumor growth was seen in the athymic nude or SCID mice, whereas the take rate was 100% and the average tumor volume at day 60 was 1,585 mm^3 (Figure 4) in the R2G2® mice.

Figure 4: Human esophageal adenocarcinoma (FLO1) tumor growth in R2G2 mice (Study A)

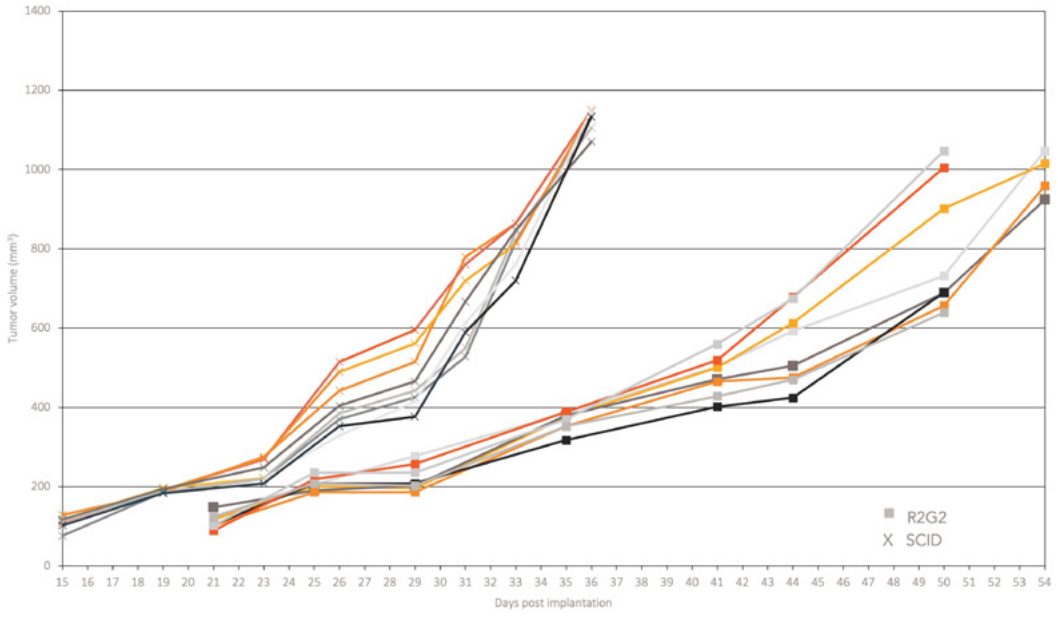


Data are represented as individual tumors

In Study B, FLO1 cells ($10x10^6$ cells per mouse) were injected into both flanks of SCID (n = 4) and R2G2® (n = 4) mice. The take rate was 100% in both the R2G2® and SCID mice, although differences were seen in growth rate.

Specifically, average tumor volume in SCID mice was 1,129 mm^3 on day 22, whereas tumors in the R2G2® mice grew slower and averaged 986 mm^3 on day 54 (Figure 5).

Figure 5: Human esophageal adenocarcinoma (FLO1) tumor growth in R2G2 mice (Study B)



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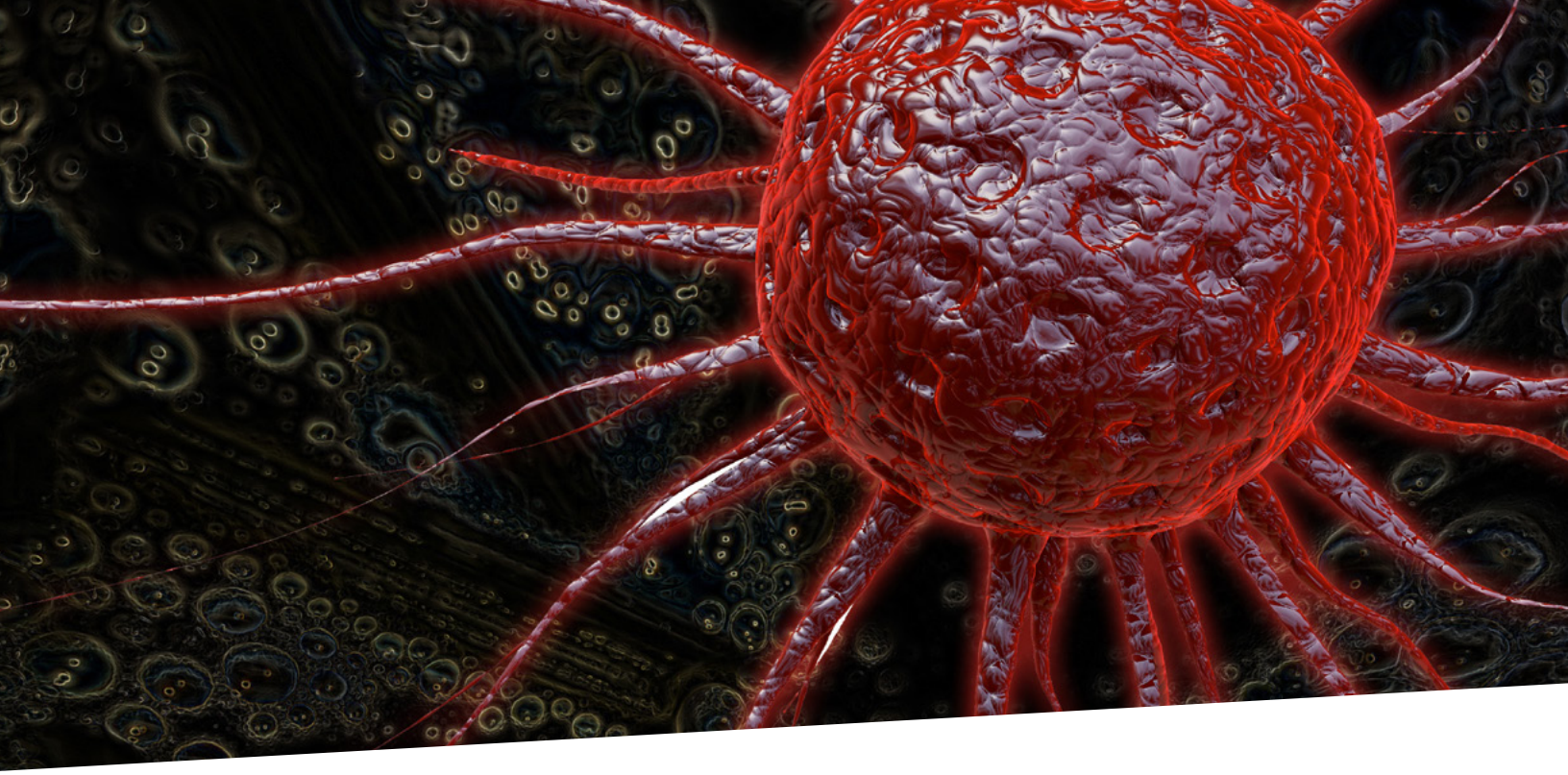
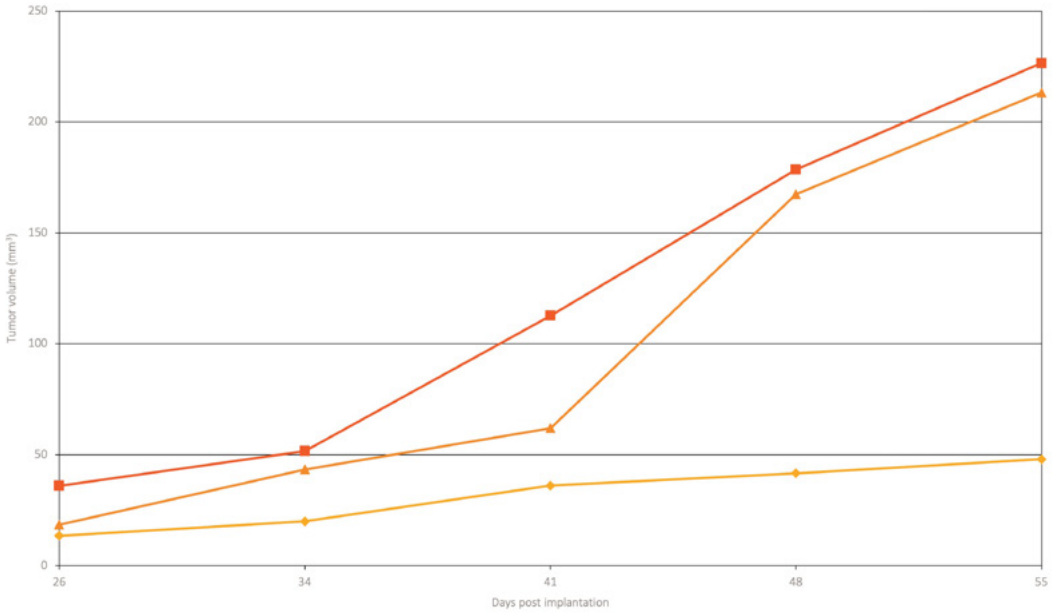


CDX: GASTRIC ADENOCARCINOMA

Human gastric adenocarcinoma AGS cells ($2x10^6$ cell per mouse) were injected into both flanks of R2G2® (n = 2) and SCID (n = 2) mice. Three of four AGS tumors developed in R2G2® mice, and no tumors were observed in SCID mice. The average tumor volume was 162.6 mm^3 on day 55 (Figure 6).

Figure 6: Human gastric adenocarcinoma (AGS) tumor growth in R2G2 mice

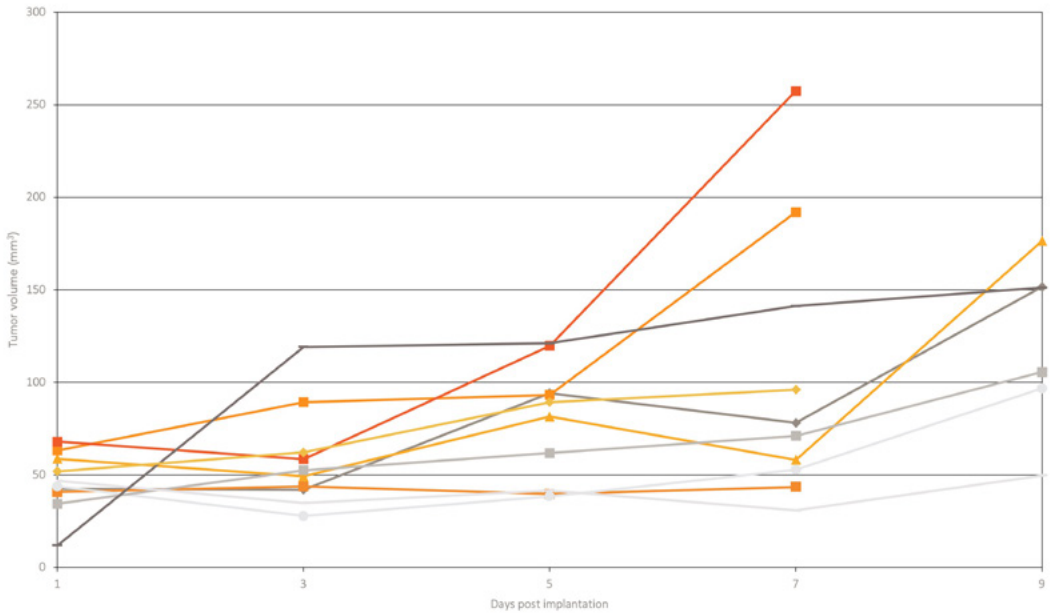


Data are represented as individual tumors

CDX: HEAD AND NECK CANCER

Head and neck squamous cell carcinoma SQ20b cells were injected into one flank of R2G2® mice (n = 10). The take rate was 90%, and the average tumor volume was 121.9 mm^3 on day 9 (Figure 7).

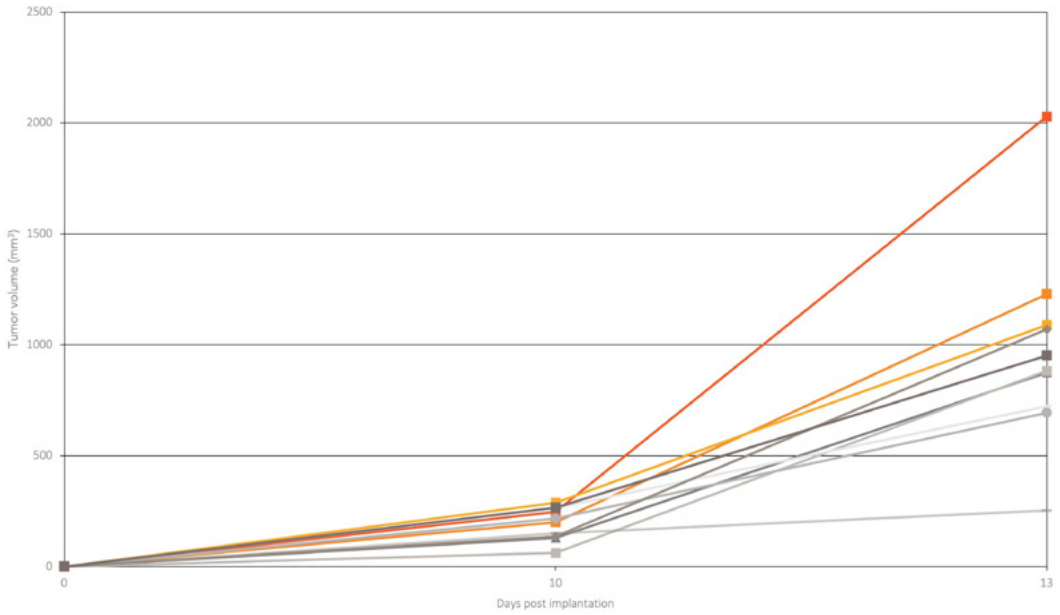
Figure 7: Human head and neck squamous cell carcinoma (SQ20b) tumor growth in R2G2 mice



ALLOGRAFT: COLON CARCINOMA

Mouse colon carcinoma CT26 cells (1x10^5 cells per mouse) were injected subcutaneously into the flanks of R2G2® mice (n = 10). The take rate was 100%, and the average tumor volume was 3,028 mm^3 on day 17 (Figure 8).

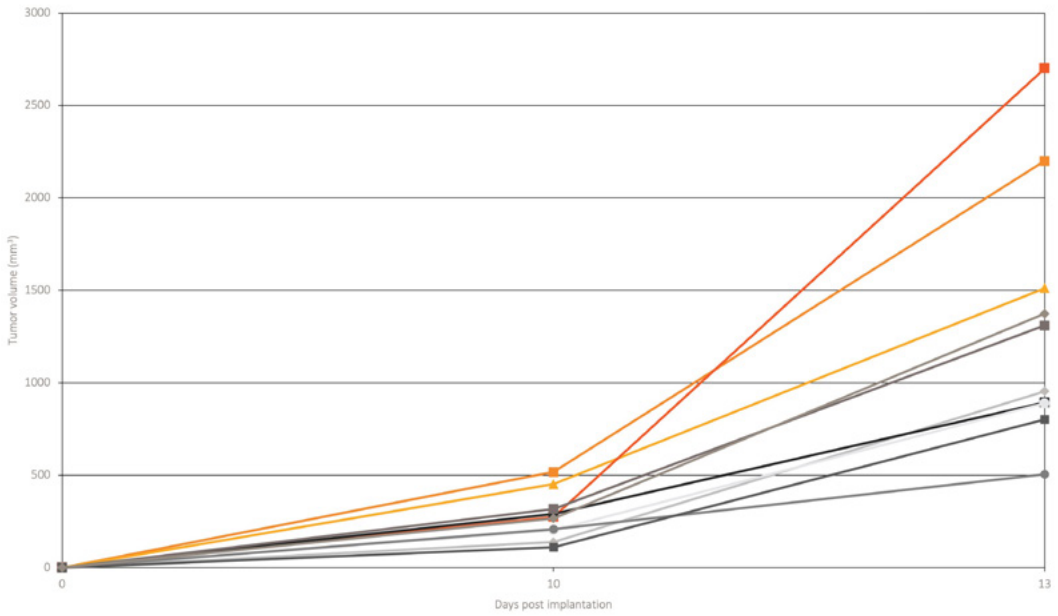
Figure 8: Mouse colon carcinoma (CT26) tumor growth in R2G2 mice



ALLOGRAFT: B-CELL LYMPHOMA

Mouse B-cell lymphoma A20 cells ($4x10^6$ cells per mouse) were injected subcutaneously into the flanks of R2G2® mice (n = 10). The take rate was 100%, and the average tumor volume was 4,101 mm^3 on day 17 (Figure 9).

Figure 9: Mouse B-cell lymphoma (A20) tumor growth in R2G2 mice



Conclusions

The R2G2® mouse is an additional tool for researchers to consider for their preclinical cancer studies. Some of its unique characteristics make it particularly suitable to generate PDX, CDX, and allograft models.

In some cases, it was shown to have higher take rates and better tumor growth rates than other commonly used immunodeficient strains, such as athymic nude or SCID mice. In addition to the reduced radiosensitivity of R2G2® (compared to NSG™) mice and the tolerance for standard-of-care chemotherapy and estrogen supplementation, the tumor growth data presented in this paper further strengthen the argument that the R2G2® mouse is a superior choice as a host in preclinical cancer studies, including those testing investigational cancer agents.

Inotiv scientists have extensive knowledge and experience in the selection of immunodeficient models that can position your preclinical study for success.

Get in touch for a free consultation that can guide your study to success.

REFERENCES

1. **Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, Zhang C, Schnell C, Yang G, Zhang Y, Balbin OA, Barbe S, Cai H, Casey F, Chatterjee S, Chiang DY, Chuai S, Cogan SM, Collins SD, Dammassa E, Ebel N, Embry M, Green J, Kauffmann A, Kowal C, Leary RJ, Lehar J, Liang Y, Loo A, Lorenzana E, Robert McDonald E 3rd, McLaughlin ME, Merkin J, Meyer R, Naylor TL, Patawaran M, Reddy A, Röelli C, Ruddy DA, Salangsang F, Santacroce F, Singh AP, Tang Y, Tinetto W, Tobler S, Velazquez R, Venkatesan K, Von Arx F, Wang HQ, Wang Z, Wiesmann M, Wyss D, Xu F, Bitter H, Atadja P, Lees E, Hofmann F, Li E, Keen N, Cozens R, Jensen MR, Pryer NK, Williams JA, Sellers WR. (2015 Nov)** High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nat Med.* ;21(11):1318-25.
2. **Goldman, J. P., M. P. Blundell, L. Lopes, C. Kinnon, J. P. Di Santo, and A. J. Thrasher. (1998).** 'Enhanced human cell engraftment in mice deficient in RAG2 and the common cytokine receptor gamma chain', *Br J Haematol*, 103: 335-42.
3. **Hong, J., Maacha, S. and Belkhiri, A., (2018).** Transcriptional upregulation of c-MYC by AXL confers epirubicin resistance in esophageal adenocarcinoma. *Molecular Oncology*, 12(12), pp.2191-2208.
4. **Jimenez-Segovia, A., Mota, A., Rojo-Sebastian, A., Barrocal, B., Rynne-Vidal, A., Garcia-Bermejo, M.L., Gomez-Bris, R., Hawinkels, L.J., Sandoval, P., Garcia-Escudero, R. and Lopez-Cabrera, M., (2018).** Prostaglandin F2α-induced TGFβ-PMEPA1 pathway is a critical mediator of epithelial plasticity and ovarian carcinoma progression. *bioRxiv*, p.418582.
5. **Johnson JI, Decker S, Zaharevitz D, Rubinstein LV, Venditti JM, Schepartz S, Kalyandrug S, Christian M, Arbuck S, Hollingshead M, Sausville EA. (2001, May 18).** Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. *Br J Cancer.* ;84(10):1424-31.
6. **Jung J. Human tumor xenograft models for preclinical assessment of anticancer drug development.** *Toxicol Res.* 2014 Mar;30(1):1-5.
7. **Langdon SP, Hendriks HR, Braakhuis BJ, Pratesi G, Berger DP, Fodstad O, Fiebig HH, Boven E. (1994 May).** Preclinical phase II studies in human tumor xenografts: a European multicenter follow-up study. *Ann Oncol.* ;5(5):415-22.
8. **Mazurier, F., A. Fontanellas, S. Salesse, L. Taine, S. Landriau, F. Moreau-Gaudry, J. Reiffers, B. Peault, J. P. Di Santo, and H. de Verneuil. (1999).** 'A novel immunodeficient mouse model--RAG2 x common cytokine receptor gamma chain double mutants--requiring exogenous cytokine administration for human hematopoietic stem cell engraftment', J Interferon Cytokine Res, 19: 533-41.
9. **Mombaerts, P., J. Iacomini, R. S. Johnson, K. Herrup, S. Tonegawa, and V. E. Papaioannou. (1992).** 'RAG-1-deficient mice have no mature B and T lymphocytes', Cell, 68: 869-77.
10. **Page, A., Ortega, A., Alameda, J.P., Navarro, M., Paramio, J.M., Saiz-Pardo, M., Almeida, E.I., Hernández, P., Fernández- Aceñero, M.J., García-Fernández, R.A. and Casanova, M.L., (2019).** IKKα promotes the progression and metastasis of non-small cell lung cancer independently of its subcellular localization. *Computational and Structural Biotechnology Journal.*
11. **Pompili L, Porru M, Caruso C, Biroccio A, Leonetti C. (2016 Dec 5). Patient derived xenografts: a relevant preclinical model for drug development.** *J Exp Clin Cancer Res.* ;35(1):189.
12. **Puck, J. M., A. E. Pepper, P. S. Henthorn, F. Candotti, J. Isakov, T. Whitwam, M. E. Conley, R. E. Fischer, H. M. Rosenblatt, T. N. Small, and R. H. Buckley. (1997).** 'Mutation analysis of IL2RG in human X-linked severe combined immunodeficiency', Blood, 89: 1968-77.
13. **'Rag2-Il2rg Double Knockout Mouse (R2G2)'. (2018).** Inotiv. https://www.inotivco.com/products-services/research-modelsservices/ models/research-models/mice/mutant/rag2-il2rgdouble-knockout-mouse-(r2g2)/.
14. **Shinkai, Y., G. Rathbun, K. P. Lam, E. M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A. M. Stall, and et al. (1992).** *'RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement',* Cell, 68: 855-67.
15. **Xu C, Li X, Liu P, Li M, Luo F. (2019 Jan).** (Patient-derived xenograft mouse models: A high fidelity tool for individualized medicine. *Oncol Lett.* ;17(1):3-10.

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