Hepatocellular Carcinoma: Animal Models Available to Characterize Tumor Immunology and Optimize Treatment Development

Gael S Roth¹,²,³, Zuzana Macek Jilkova¹,²,³, Thomas Decaens¹,²,³*

¹Université Grenoble Alpes, France
²Institute for Advanced Biosciences, Research Center UGA / Inserm U 1209 / CNRS 5309, Grenoble, France
³Clinique Universitaire d’Hépato-gastroentérologie, Pôle Digidune, CHU Grenoble Alpes, France

*Correspondence should be addressed to Thomas Decaens; tdecaens@chu-grenoble.fr

Received date: August 07, 2020 Accepted date: August 27, 2020

Copyright: © 2020 Roth GS, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Hepatocellular carcinoma is the second cause of cancer-related death worldwide with few therapeutic options. In this field, the rate of bench-to-bed translation of drugs is very modest, with 70% of drug attrition suggesting that preclinical evaluation should be urgently improved. Besides, recently immunotherapy showed very promising results and constitutes a real breakthrough in the area of HCC treatment. As these treatments aim to restore anti-tumor immunity, animal models including a functioning immune system are essential to allow a relevant preclinical validation. Macek Jilkova et al. describe, in a clear and forceful review which animal models can optimize this research and what are the pros and cons of each of them.

Keywords: Hepatocellular carcinoma, Liver immunology, Animal models, Preclinical drug study

Introduction

Hepatocellular carcinoma (HCC) is the second cause of cancer-related death worldwide with almost 1 million new cases per year. At the diagnosis, 70% of patients have only access to a palliative treatment [1,2] with few therapeutic options mostly represented by tyrosine kinase inhibitors such as sorafenib [3] and lenvatinib [4] in first line; regorafenib [5] and cabozantinib [6] in second line. HCC occurs on a cirrhotic liver in more than 90% of cases and liver is a singular organ from an immunological point of view. Cirrhosis modulates liver immune landscape through chronic alterations such as inflammation and fibrosis and these immune changes may induce aberrant immunotolerance through the activation of multiple pathways involving major immune functions such as antigen presentation or lymphocytes’ exhaustion. These modifications lead to failure to immunosurveillance systems and allow tumor initiation and progression [7]. For that reason, HCC seems to be a good candidate to immunostimulatory therapies aiming to restore anticancer immunity. These therapies are currently strongly studied in this pathology with the advent of monoclonal antibodies directed against immune checkpoints, especially against PD-1/PD-L1 pathway. Two new combination therapies particularly stand out: atezolizumab (anti-PD-L1) - bevacizumab (anti-angiogenic) which is becoming the new standard in first line [8] and durvalumab (anti-PD-L1) - tremelimumab (anti-CTLA4) [9]. Nonetheless, a large proportion of patients do not respond to these treatments and complex physiopathological mechanisms involved in HCC oncogenesis, as well as resistance pathways activated during these immunotherapies are still poorly understood.

Early Drug Development Phases in HCC: A Major Challenge

New therapies are urgently needed, and the use of combinations targeting multiple pathways seems to be the most promising approach. However, due to cumulative toxicities, preclinical phases are essential before testing these innovative therapeutic schemes in humans. To this day, the rate of bench-to-bed translation of candidate anticancer drugs is very modest, with the failure in clinical
phases of approximately 70% of drugs initially considered as hopeful based on preclinical data [10,11]. Consequently, preclinical drug studies need to be improved to optimize drug selection and better study their efficacy and safety in a relevant manner. Indeed, several issues are usually encountered in classical drug development using inadequate animal models such as xenograft. These models are useful in large-scale drug testing because they are cheap, and a rapid- and easy-to-assess tumor growth, but they present many limits [12]. Mainly, xenograft models do not reproduce tumor heterogeneity. In fact, they present an irrelevant tumor microenvironment due to a non-functional immune system and the absence of organ chronic alterations preceding cancer such as cirrhosis, in the case of HCC [13,14]. The absence of underlying cirrhosis in the vast majority of animal models used in preclinical studies related of HCC, is probably one of the main causes of the high rate of drug attrition, and this concerns either cytotoxic chemotherapies, target therapies, or immunotherapies. Finally, recent studies suggest that many drugs tested in humans are probably targeting superfluous pathways, due to an insufficient knowledge of true driver genes involved in cancers [15]. In all, these elements suggest that specific animal models are needed to help research in the field and optimize anticancer drug development.

Animal Models Available to Better Characterize HCC Immunology and Optimize Drug Selection

Macek Jilkova et al. proposed a well-documented and illustrated review detailing the most relevant animal models available to study HCC immunology and immunotherapies [28]. Models include generation of tumors by chemical agents, the use of genetically engineered mice (GEM), or utilization of syngeneic and humanized animals. Each model presents advantages and limits summarized in Table 1; however, it is important to mention that every model remains artificial for various reasons.

For example, chemically induced models using carcinogens such as diethylnitrosamine (DEN) are interesting as they reproduce the physiopathological sequence of chronic inflammation-fibrosis-cirrhosis preceding cancer [16-19]. The DEN model also is relevant because it keeps a functional immune system. Nonetheless, DEN is a strong

| Animal models in HCC | Advantages | Disadvantages |
|----------------------|------------|---------------|
| Chemically induced [16-19] | Fully functional immune system | Delay of tumor induction |
| | Respect of inflammation-fibrosis-cancer sequence | High mutational burden differing from most human situations |
| Genetically engineered [20] | Specific study of an oncogene | Overrepresentation of mutated oncogenes/tumor suppressors |
| | Fully functional immune system | Lack of inflammation-fibrosis-cancer sequence |
| Syngeneic [21-23] | Fully functional immune system | Limited similarity to human HCC |
| | Metastasis formation | Lack of inflammation-fibrosis-cancer sequence |
| Xenograft [13,14] | Low cost and rapid | Lack of inflammation-fibrosis-cancer sequence |
| | Easy to measure tumor progression | Lack of functioning immune system |
| Humanized [24-27] | Functional immune system with presence of specific actors of human cancer immunology | High cost, high level of expertise and high-tech facility |
| | Particularly relevant to study immunotherapy | Lack of inflammation-fibrosis-cancer sequence |

Table 1: Summary of different animal models available to study anticancer drugs efficacy and consequences.
carcinogen which induces multiple DNA alterations that do not correspond precisely to the mutational landscape of the majority of human HCCs.

Models such as GEM allow for a better study of the impact of a specific genetic alteration and are developed in mice with a functional immune environment as well. These models are particularly interesting to study the driver genes and the main pathways involved in tumor initiation and progression. For example, the transgenic AlbLTαβ model with aberrant expression of the cytokine lymphotixin helped to better characterize inflammation-induced hepatocarcinogenesis [20]. Nevertheless, GEM suffer from a low and stereotypic mutational burden, and they usually do not present the inflammation-fibrosis-cirrhosis sequence. Also, even though their development duration can be shortened through accumulation of several mutations, or the use of new techniques such as CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9, these models stay time-consuming, expensive, and require large infrastructures.

Other models that are particularly interesting to reproduce the complexity of the tumor microenvironment in mice with full functioning immune system are the syngeneic models. They consist of the injection in immunocompetent mice, of tumor cells derived from the same species, directly in the organ which is concerned by the cancer being studied. They offer the possibility to study all key players of the immune system and tumor microenvironment, including tissue architectural considerations such as the vasculature, the stroma, and the surrounding lymphatic system [21]. Another advantage is their potential to develop metastases, which is particularly relevant to study concepts such as tumor invasion or metastatic homing, and their relation with tumor immune microenvironment [22]. However, one of the most important limits of these models resides in their mutational landscape that differs from humans with different driver genes involved in the tumorigenesis process. Moreover, as the immune system is still functional in these animals, the implication of aberrant immunotolerance in tumorigenesis cannot be observed and may lead to spontaneous tumor regression, which increases the distance with the human situation [23].

Thus, the development of models better mimicking HCC pathogenesis in human with realistic tumor-immune system interactions represents a major challenge to improve drug study. Humanized animal models constitute a very valuable tool to study these elements. Indeed, they are based on the engraftment of human immune cells such as peripheral blood lymphocytes or CD34+ hematopoietic stem cells, in severe combined immunodeficient rodents [24-26], followed by tumor xenographs such as patient derived xenographs [27]. Humanized models are very attractive to improve anticancer drug development such as immunotherapy as they reproduce the complexity and specificity of humans HCC and their interactions with immune system. Humanized mice, however are still poorly used in HCC field, mostly due to their high cost and the high level of technicity that they require.

Conclusion

Oncology research is constantly evolving with many new candidate therapies which are more and more focused on specific pathways involved in multiple mechanisms such as tumor progression, angiogenesis or tumor-immune system interactions. Besides, the high level of drug attrition from the bench to the early clinical phases, strongly suggest that the use of old models such as basic xenographs models is not appropriate. Thus, the use of innovative models that respect crucial principles present in HCC such as a strong tumor heterogeneity, the presence of an underlying cirrhosis and a complex and singular immune system, is essential and should be systematic to develop and assess future anticancer drugs in a relevant manner. The review proposed by Macek Jilkova et al. offers a very clear overview of preclinical models available to study liver immunology and its implications in HCC, by demonstrating the pros and cons of each animal model.

References

1. European Association. European Association For The Study Of The L, European Organisation For R, Treatment Of C. EASL EORTC clinical practice guidelines: management of hepatocellular carcinoma. J. Hepatol. 2012;56(4):908-43.

2. Llovet, J. M. et al. Hepatocellular carcinoma. Nat Rev Dis Primers 2, 16018, doi:10.1038/nrdp.2016.18 (2016).

3. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. New England journal of medicine. 2008 Jul 24;359(4):378-90.

4. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, Blanc JF. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. The Lancet. 2018 Mar 24;391(10126):1163-73.

5. Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Breder V, Gerolami R. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESOURCE): a randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet. 2017 Jan 7;389(10064):56-66.
Roth GS, Jilkova ZM, Decaens T. Hepatocellular Carcinoma: Animal Models Available to Characterize Tumor Immunology and Optimize Treatment Development. J Cancer Immunol. 2020; 2(4): 133-137.

6. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, Cicin I, Merle P, Chen Y, Park JW, Blanc JF, Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. New England Journal of Medicine. 2018 Jul 5;379(1):54-63.

7. Roth GS, Decaens T. Liver immunotolerance and hepatocellular carcinoma: Patho-physiological mechanisms and therapeutic perspectives. European Journal of Cancer. 2017 Dec 1;87:101-12.

8. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, Li D. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. New England Journal of Medicine. 2020 May 14;382(20):1894-905.

9. Abou-Alfa GK, Chan SL, Furuse J, Galle PR, Kelley RK, Qin S, Armstrong J, Darilay A, Vlahovic G, Negro A, Sangro B. A randomized, multicenter phase 3 study of durvalumab (D) and tremelimumab (T) as first-line treatment in patients with unresectable hepatocellular carcinoma (HCC): HIMALAYA study.

10. Bruix J, da Fonseca LG, Reig M. Insights into the success and failure of systemic therapy for hepatocellular carcinoma. Nature Reviews Gastroenterology & Hepatology. 2019 Aug 1;11.

11. Ruggeri BA, Camp F, Miknyczaki S. Animal models of disease: pre-clinical animal models of cancer and their applications and utility in drug discovery. Biochemical pharmacology. 2014 Jan 1;87(1):150-61.

12. Heindryckx F, Colle I, Van Vlierberghe H. Experimental mouse models for hepatocellular carcinoma research. International journal of experimental pathology. 2009 Aug;90(4):367-86.

13. Kung AL. Practices and pitfalls of mouse cancer models in drug discovery. Advances in cancer research. 2006 Jan 1;96:191-212.

14. Sammamed MF, Chester C, Melero I, Kohrt H. Defining the optimal murine models to investigate immune checkpoint blockers and their combination with other immunotherapies. Annals of Oncology. 2016 Jul 1;27(7):1190-8.

15. Lin A, Giuliano CJ, Palladino A, John KM, Abramowicz C, Yuan ML, Sausville EL, Lukow DA, Liu L, Chait AR, Galluzzo ZC. Off-target toxicity is a common mechanism of action of cancer drugs undergoing clinical trials. Science Translational Medicine. 2019 Sep 11;11(509):eaaw8412.

16. Uehara T, Pogribny IP, Rusyn I. The DEN and CCL4-induced mouse model of fibrosis and inflammation-associated hepatocellular carcinoma. Current protocols in pharmacology. 2014 Sep;66(1):14-30.

17. Schiffer E, Housset C, Cacheux W, Wendum D, Desbois-Mouthen C, Rey C, Clergue F, Poupon R, Barbu V, Rosmorduc O. Gefitinib, an EGFR inhibitor, prevents hepatocellular carcinoma development in the rat liver with cirrhosis. Hepatology. 2005 Feb;41(2):307-14.

18. Roth GS, Jilkova ZM, Kuyucu AZ, Kurma K, Pour ST, Abbadessa G, Yu Y, Busser B, Marche PN, Leroy V, Decaens T. Efficacy of AKT inhibitor ARQ 092 compared with sorafenib in a cirrhotic rat model with hepatocellular carcinoma. Molecular Cancer Therapeutics. 2017 Oct 1;16(10):2157-65.

19. Jilkova ZM, Kuyucu AZ, Kurma K, Pour ST, Roth GS, Abbadessa G, Yu Y, Schwartz B, Sturm N, Marche PN, Hainaut P. Combination of AKT inhibitor ARQ 092 and sorafenib potentiates inhibition of tumor progression in cirrhotic rat model of hepatocellular carcinoma. Oncotarget. 2018 Feb 16;9(13):11145.

20. Haybaeck J, Zeller N, Wolf MJ, Weber A, Wagner U, Kurrer MO, Bremer J, Iezzi G, Graf R, Clavien PA, Thimme R. A lymphotxin-driven pathway to hepatocellular carcinoma. Cancer cell. 2009 Oct 6;16(4):295-308.

21. Brown ZJ, Heinrich B, Greten TF. Mouse models of hepatocellular carcinoma: an overview and highlights for immunotherapy research. Nature Reviews Gastroenterology & Hepatology. 2018 Sep;15(9):536-54.

22. Reiberger T, Chen Y, Ramjiawan RR, Hato T, Fan C, Samuel R, Roberge S, Huang P, Lauwers GY, Zhu AX, Bardeesy N. An orthotopic mouse model of hepatocellular carcinoma with underlying liver cirrhosis. Nature protocols. 2015 Aug;10(8):1264.

23. Buijs M, Geschwind JF, Syed LH, Ganapathy-Kanniappan S, Kunjithapatham R, Wijlemans JW, Kwak BK, Ota S, Vali M. Spontaneous tumor regression in a syngeneic rat model of liver cancer: implications for survival studies. Journal of Vascular and Interventional Radiology. 2012 Dec 1;23(12):1685-91.

24. Kamel-Reid S, Dick JE. Engraftment of immuno-deficient mice with human hematopoietic stem cells. Science. 1988 Dec 23;242(4886):1706-9.

25. Mosier DE, Gulizia RJ, Baird SM, Spector S, Spector D, Kipps TJ, Fox RI, Carson DA, Cooper N, Richman DD, Wilson DB. Studies of HIV infection and the development of Epstein-Barr virus-related B cell lymphomas following transfer of human lymphocytes to mice with severe combined immunodeficiency. InThe Scid Mouse 1989 (pp. 195-199). Springer, Berlin, Heidelberg.
26. De La Rochere P, Guil-Luna S, Decaudin D, Azar G, Sidhu SS, Piaggio E. Humanized mice for the study of immuno-oncology. Trends in immunology. 2018 Sep 1;39(9):748-63.

27. Zhao Y, Shuen TW, Toh TB, Chan XY, Liu M, Tan SY, Fan Y, Yang H, Lyer SG, Bonney GK, Loh E. Development of a new patient-derived xenograft humanised mouse model to study human-specific tumour microenvironment and immunotherapy. Gut. 2018 Oct 1;67(10):1845-54.

28. Macek Jilkova Z, Kurma K, Decaens T. Animal models of hepatocellular carcinoma: the role of immune system and tumor microenvironment. Cancers. 2019 Oct;11(10):1487.