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Adapting cancer immunotherapy models for the real world

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Abstract

Early experiments in mice predicted the success of checkpoint blockade immunotherapy in cancer patients. However, these same animal studies failed to accurately predict many of the limitations and toxicities of treatment. One of the likely reasons for this discrepancy is the nearly universal use of young healthy mice, which stand in stark contrast to diverse patient populations varying in age, weight, diet and hygiene. Because these variables impact immunity and metabolism, they also influence outcomes during immunotherapy and should be incorporated into the study design of preclinical experiments. Here, we discuss recent findings that highlight how efficacy and toxicity of cancer immunotherapy are affected by patient variation, and how distinct host environments can be better modeled in animal studies.

Keywords

cancer; immunotherapy; checkpoint blockade; age; obesity; microbiota

Host factors dictate success of cancer immunotherapy

Immunotherapy with checkpoint blockade antibodies has forever changed the outlook for many patients with cancer [1-5]. However, only a subset of patients respond to this promising treatment, and toxicities are common [6]. The challenge now is to extend the range of patients that benefit from immunotherapy while minimizing treatment-related adverse events. This endeavor raises new questions fundamental for improving cancer immunotherapy. What biological mechanisms shape a patient's response to treatment? Are these mechanisms useful biomarkers to predict which patients will benefit versus those at highest risk of harm? Answering these questions requires fresh insight into the host factors that dictate such divergent outcomes.

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One of the obstacles to discovery of predictive biomarkers is the study of animal models that fail to recapitulate human cancer patients. For example, cancer is most often associated with aging. The American Cancer Society reported that less than 10% of new cancer cases in 2013 occurred in people under the age of 45, whereas more than 50% occurred after age 65. Another influential factor is obesity. According to the CDC, more than 78 million (~35%) adult Americans are obese, and the prevalence continues to grow [7]. Combined with an elevated incidence of cancer among obese people, this all but ensures high numbers of obese cancer patients in the future [8]. Additionally, our commensal microbiota may contribute to malignancy [9-11]. Despite these revelations, the majority of preclinical immunotherapy research has been performed in young, lean and inbred mice. These animals either do not experience the same level of toxicity or variability seen in human patients, or such issues are ignored and go unreported. To overcome these limitations, a handful of recent studies have used models that reflect important aspects of patient variability in mice receiving immunotherapy [12-16]. They report disparate outcomes associated with increased age, obesity and microbiota composition. Thus, it is tempting to speculate that these factors also influence outcomes in cancer patients being treated with immunotherapy and warrant rigorous examination. Of course, the influences of age, obesity and microbiota are not mutually exclusive, and all likely impact a range of immune responses in diverse patient populations. The complexity of these important elements necessitates the rational diversification of animal models to reflect challenges faced in the clinic. Below, we review new findings that provide a roadmap for the design of more informative preclinical immunotherapy studies.

Considering age when testing cancer immunotherapies

Immunotherapy with ipilimumab and nivolumab (anti-CTLA-4 and anti-PD-1) in melanoma patients averaging 58-65 years old achieved objective responses in more than half of subjects participating in three separate clinical trials [2, 3, 17]. For many though, the cost of boosting antitumor immunity was treatment-related toxicity, forcing discontinuation of treatment in approximately 40% of patients. Upon subsequent analysis, Bristol-Myers Squibb has since reported that the hazard associated with this combination immunotherapy increased with patient age [18], but the mechanisms behind this link remain undefined. Because most cancer patients are beyond 60 years of age, it can be convincingly argued that all preclinical studies testing novel immunotherapies or new combinations should include older mice. Yet, this approach is rarely taken. Aging impacts immunity in several ways. One hallmark of immune aging in humans and mice is thymic involution, leading to loss of thymic output, reduced T cell diversity and potential gaps in the T cell repertoire [19-21]. This compromises direct T cell-mediated antitumor immunity and indirect T cell-dependent processes such as the production of antitumor antibodies [22]. At the same time, age-related adiposity induces chronic low-grade inflammation mediated mostly by macrophages. Monocytes and macrophages are recruited to adipose tissue via CCR2-dependent migration where they elevate expression of proinflammatory cytokines like TNF, IL-6 and IL-1 β [12, 23, 24]. Indeed, TNF and IL-6 production by macrophages is markedly increased in both mice and humans later in life [12]. But TNF seems to be the dominant cytokine regulating toxicity during immunotherapy, as neutralizing TNF in mice minimized liver pathology and

reduced IL-6 production after treatment with anti-CD40 and IL-2, thereby restoring therapeutic benefit [12]. This outcome highlights the pleiotropic role of TNF, but also implies that the function and diversity of T cells in aged individuals are sufficient to mount a robust antitumor immune response if deleterious inflammation is curtailed. Similar conclusions were reached when aged mice bearing a B16 melanoma were depleted of myeloid suppressor cells [25], and further supported by studies demonstrating that tumor-reactive CD8⁺ T cells in aged mice have no intrinsic defects [26]. However, the relative quality of T cells from aged compared to young mice remains controversial as the opposite conclusions were reached in other settings [27, 28].

Earlier reports in aged mice had shown reduced antitumor immune responses when studying endogenous polyclonal T cells, and interpreted as an intrinsic defect in aged T cells [29, 30]. But possible contributions by defective antigen presenting cells (APC) or the impact of reduced T cell numbers in old mice were not completely ruled out, and could easily be misinterpreted as intrinsic T cell dysfunction. This led to experiments employing equivalent numbers of clonal tumor-reactive CD8⁺ T cells expressing a transgenic T cell receptor (TCR), where no defect in APC function, T cell proliferation or antitumor immunity was associated with advanced age [26]. However, these data are in conflict with several subsequent studies of CD4⁺ and CD8⁺ T cells from older mice demonstrating poor responses to either *in vitro* stimulation with peptide or anti-CD3 or *in vivo* encounter with virus [27, 28], which has been reviewed elsewhere [21]. The reason for these discrepancies is not fully understood, but experimental design (tumor versus infection), mouse strain, TCR affinity, antigen presentation and even differences in microbiota could account for the variation. While most agree that intrinsic T cell defects affect immunity as we age, the mechanisms regulating this phenomenon have not been defined, particularly for CD8⁺ T cells. Solving this puzzle is especially relevant for cancer immunology and immunotherapy where patient age is usually advanced and correlated with outcomes. Despite decades of research into immune senescence during aging, this still represents fertile territory for clinically significant discoveries. Therefore, the direct comparisons of old and young mice in translational preclinical investigation is crucial moving forward.

Modeling old age in mice

The lifespan of mice commonly used in research settings can vary significantly. C57BL/6J (B6) mice are relatively long-lived, with an average lifespan of approximately 884 days (2.4 years or 29 months), whereas BALB/c mice live for only 732 days (2 years or 24 months) on average [31]. To model the aging human immune system in mice, preclinical researchers have routinely used mice between 16-24 months of age [12, 20, 27, 32]. These are chronologically equivalent to U.S. citizens at 43-66 years old based on the average life expectancy of 79 years according to the Centers for Disease Control (CDC). While the earlier years of this range are not typically considered old for humans, it is important to remember that the chronological age of two different species does not necessarily mirror their phenotypic age. At 16-24 months, mice recapitulate the thymic atrophy, chronic inflammation, and increased adiposity observed in older humans [12, 19, 33, 34]. Importantly, one study showed toxicity related to immunotherapy increased significantly in mice as young as 9 months, whereas no toxicity was observed in mice younger than 6

months [12]. Thus, older mice represent imperfect but valuable models for human immune aging, and may prove far more accurate than young mice in predicting the efficacy and potential toxicity of novel cancer immunotherapies in this major patient demographic.

A confounding issue when thinking about how age impacts tumor immunology in mouse models is the nature of the tumor being studied. Transplanted tumors can be established in mice at any age, allowing direct comparisons of tumor immunity in old and young mice. However, one of the limitations to tumor transplantation is that it does not mimic the initiation phase and gradual progression of naturally arising cancer [35]. To overcome this, some researchers are exploring genetically engineered mouse models (GEMM), where tumors are induced from endogenous tissue through engineered overexpression of an oncogene or loss of a tumor suppressor gene [36, 37]. But these require time for autochthonous tumors to develop, thus there is an inherent increase in the minimal age of such mice upon evaluation. This has the potential to confound interpretation of immune responses against cancer, but perhaps better reflects the age of the patient population being modeled. Regardless, the inability to easily control age as a variable in these mice requires careful consideration when designing and interpreting immunotherapy studies in GEMM. Another potential caveat to studying immune responses in older mice is the possible influence of endogenous retroviruses (ERVs). As mice age, regions of the murine genome containing endogenous retroviruses can become demethylated, leading to reactivation [38]. Reactivated ERVs induce inflammation as part of an anti-viral response involving production of interferons [39, 40]. This aberrant hypomethylation can also promote neoantigen expression by tumors and, when coupled with changes in inflammation, can significantly influence immune responses to cancer. Indeed, intentional reactivation of ERVs using demethylating chemotherapy agents is being evaluated as a strategy to enhance immunotherapy for treatment of cancer [41].

Obesity influences immunity

Obese individuals display many of the same immunological abnormalities seen in old age, stemming from chronic inflammation and increased adiposity [13, 42-44]. The thymic involution that undermines immunity as humans age is facilitated by accumulation of adipose tissue (fat) in the thymus [45, 46], and obesity hastens the rate of thymic atrophy [47]. At the foundation of all adipose tissue is the adipocyte, which serves a broad spectrum of biological functions important for mammalian health, energy storage, metabolism and reproduction [8]. During progression toward obesity, individual adipocytes grow in volume. This increased size, rather than increased adipocyte numbers, largely accounts for excess fat mass in obese people [48]. Unchecked caloric excess eventually leads to adipose tissue remodeling as the growth of fat depots outpace new blood vessel formation, inducing hypoxia, apoptosis and inflammation [49]. While adipocytes make up most of volume in adipose tissue, they are often outnumbered by cells of the immune system, including T cells, B cells and macrophages [24, 50, 51]. The frequencies and phenotypes of these immune cells regulate inflammation during obesity, which corresponds with a loss of Foxp3⁺ regulatory CD4⁺ T cells and the simultaneous influx of CD8⁺ T cells and inflammatory macrophages into adipose tissues [42, 50, 52]. Ultimately, large masses of

adipose tissue in obese individuals become complex inflammatory sites with broad effects on metabolism and systemic immune responses.

While the exact mechanisms connecting adiposity-induced inflammation with immune system disruption are unknown, the impact on cellular immunity is clear. Studies have shown reduced T cell proliferation and effector activity in obese mice, but these results are uniformly attributed to impaired dendritic cell (DC) function, not intrinsic T cell defects [13, 43]. Moreover, the generation and maintenance of virus-specific CD8⁺ memory T cells appears normal in obese mice despite high levels of TNF in the serum [53]. Thus, unlike in aged mice, T cells in obese mice have no obvious intrinsic defect. Altered T cell subset frequencies have been observed in obese people [54]. In contrast, DC frequency is slightly elevated in spleens of obese mice, but these APCs fail to efficiently stimulate T cells relative to lean control mice despite equivalent expression of major histocompatibility (MHC) molecules and costimulatory ligands [13, 43]. Poor T cell activation in obesity also impedes T cell-dependent B cell functions like production of specific serum IgG in response to foreign antigen and vaccination [43, 55]. Clearly, impaired DC function has the capacity to disrupt a plethora of immune responses in obese individuals, with obvious implications in tumor immunology.

Obesity and immunotherapy

The increased prevalence of cancer in obese people is well-established and likely stems in part from a lack of vigilant immune surveillance [56-58]. Research into the safety and utility of immunotherapy for obese cancer patients has been sparse. In clinical trials that identified age as a risk factor for toxicity during checkpoint blockade immunotherapy, increased body weight did not correlate with efficacy or toxicity [18]. But total body weight does not necessarily reflect obesity, which is more accurately measured by the percentage of visceral adipose tissue, body mass index, or the waist-to-height ratio [59]. These metrics were not included as covariates for safety, leaving unresolved whether obesity contributes to either the positive or negative effects of immunotherapy.

Research into how obesity impacts the success of cancer immunotherapy has been sparse, and the results of two recent studies are illustrated in Figure 1. In a mouse model of renal cell carcinoma, immunosuppressive DCs were shown to infiltrate tumors and inhibit IFN γ -producing effector CD8⁺ T cells. Combination therapy with an adenoviral vector expressing TRAIL (intended to induce tumor cell apoptosis) and inflammatory CpG (intended to promote DC maturation) reduced tumor burden in normal weight mice but not obese recipients [13]. Because this treatment relies on DCs to present tumor antigen to CD8⁺ T cells, this shortcoming was attributed to excessive tumor infiltration by inhibitory DCs in obese mice (Fig. 1A). These data suggest immunotherapies targeting the activity of DCs are likely to be ineffective in obese patients. It is important to note that no toxicities were reported in these obese tumor-bearing mice treated with Ad-TRAIL/CpG. This is in sharp contrast to obese mice receiving either anti-CD40 or CpG along with high-dose IL-2, which produced a lethal cytokine storm in obese mice within just days of treatment, whereas all normal weight mice survived the same immunotherapy regimen [14]. Obese mice had elevated levels of serum TNF and IL-6 proinflammatory cytokines following treatment, as

well as inflammatory tissue damage in the liver and intestines (Fig. 1B). This severe toxicity was entirely attributable to TNF-produced by macrophages in the peritoneum and visceral fat of obese mice. Deletion of these macrophages with liposomal clodronate or blocking TNF with etanercept (Enbrel) completely protected obese mice given anti-CD40/IL-2 immunotherapy. This study dramatically illustrates how obesity can place individuals at increased risk for harm during immunotherapy, and provides insight into strategies to mitigate this risk by limiting inflammation. Unfortunately, the severe toxicity associated with this treatment prevented assessment of antitumor immunity in these obese mice, and no tumor studies were performed in mice treated with protective clodronate or etanercept. This leaves open the question of how effective cancer immunotherapy could be in obese patients, particularly when using checkpoint blockade antibodies that primarily engage effector T cells and natural killer cells rather than macrophages and DCs. Certainly, checkpoint blockade carries a risk of immune-mediated toxicity [2, 3, 6, 17]. Determining whether these adverse events are exacerbated in obese patients, and whether such toxicity can be managed with targeted interventions requires new studies using animal models of obesity.

Obese mice: What are we modeling?

Strategies for generating obese research mice involve manipulation of genetics or diet. Mice on the C57BL/6 background lacking the leptin gene (B6.Cg-*Lep^{ob}/J* or *ob/ob*; commercially available from JAX) are commonly studied as a model of human morbid obesity [60]. One function of the leptin hormone is control of appetite. Without leptin, mice gain weight partly due to excessive eating of normal chow, but leptin also has other roles in adipose physiology and metabolism [61]. Because human obesity is primarily the result of excessive calorie consumption, many researchers prefer models of diet-induced obesity (DIO). These act more slowly and induce obesity over a period of several months by providing chow that is very high in fat (60% kcal), compared to normal chow that is typically less than 10% fat (Table 1). One important consideration here is that DIO requires time, and these obese mice will be older when they are studied, bringing age into play as a potential variable. The only study to directly compare these different obesity models during immunotherapy reported lethal toxicity induced by anti-CD40/IL-2 to a similar extent in obese *ob/ob* leptin knockout mice and in DIO mice C57BL/6 mice on a high-fat diet [14].

Mouse strain is an important factor when planning DIO projects. BALB/c are heterogeneously resistant, and after 20 weeks on a high-fat diet (D12492 from Research Diets, Inc.) only 45-55% of BALB/c weigh significantly more than normal diet controls [13]. C57BL/6 mice experience more uniform weight gain and become significantly heavier than control mice by 8-10 weeks [62, 63]. In these models, fat calories drive obesity, but the causative role of dietary fat in human obesity is probably only partial. Other dietary components should be considered when modeling human eating habits that lead to obesity and immune dysfunction. For example, excessive consumption of fructose found in soft drinks and processed foods is associated with accumulation of visceral fat in adolescents 14-18 years old [64]. This clear path to adult obesity is part of many people's routine diet, but is rarely if ever considered in DIO studies of cancer or immunology. One option is the non-alcoholic steatohepatitis (NASH) diet (D09100301 from Research Diets, Inc.), which is high in fat (45% kcal, 40% kcal from trans-fats) and in fructose and sucrose [65] (Table 1).

The NASH diet induces weight gain from the accumulation of adipose tissue accompanied by systemic inflammation involving TNF and IL-6 similar to other obesity model, and may more accurately reflect the poor diet and relevant health complications seen in some obese patients.

Microbiota and immune responses to cancer

When the human genome was mapped at the beginning of the 21st century, it was anticipated that this information would unlock the secrets to human health. It is now appreciated that most diseases are far more complex than the genes encoded within our DNA sequence. While mutations in human genes can certainly cause disease and predispose some individuals to cancer, recent studies have also discovered contributions by our “other genome,” existing within the microbes that colonize the human body (microbiota). The links between commensal bacteria and malignancy have been established but are still poorly understood [9-11].

Microbial colonization of a human fetus begins *in utero* [66-68], and is further enriched during birth and even biased by delivery method. Vaginal delivery is associated with colonization by bacteria from the vaginal mucosa, whereas skin microbes dominate the infant microbiota after cesarean section [69]. Microbes continue to influence metabolism and immunity throughout life, and this topic has been thoroughly reviewed elsewhere [70]. Here, we focus on how our microbiota impact immune responses to cancer, which can hinder or help tumor progression (Fig. 2). For example, total body irradiation leads to increased serum LPS and translocation of bacteria to mesenteric lymph nodes, enhancing the antitumor activity of adoptively transferred tumor-reactive CD8⁺ T cells through TLR4 signaling [71]. In contrast, IL-23 production by tumor-associated myeloid cells activated by microbial products can trigger an IL-17 response that drives tumor growth in a model of colorectal adenoma [72]. It was recently shown that genetically similar C57BL/6 mice obtained from two different colonies (Jackson Laboratory and Taconic Farms) mounted distinct immune responses against B16 melanoma, exclusively as a result of variations in gut microbiota [15]. The inextricable relationship between host and microbiota demands continued research in carefully selected models to address the role commensals play during cancer immunotherapy.

Cancer immunotherapy is influenced by microbiota

Cancer treatment with radiation, or chemotherapy with cyclophosphamide or oxaliplatin, derive some of their benefit from the microbiota, which consequently stimulates immune responses [9, 10, 71]. This is a departure from canonical mechanisms of chemotherapy where fast growing tumor cells accumulate mutations more quickly than surrounding normal cells, and are selectively killed. There is now emerging evidence that immunotherapy with checkpoint blockade antibodies relies on distinct commensal microbes (Fig. 2). The presence of *Bifidobacterium* in the gut of tumor-bearing mice promotes anti-PD-L1 efficacy through enhanced CD8⁺ T cell priming, which was eliminated upon cohousing or fecal microbial transplant (FMT) [15]. In a similar study, *Bacteroides* and *Burkholderia* contributed to anti-CTLA-4 immunotherapy and transfer of *B. fragilis*-specific T cells or *B.*

fragilis-derived polysaccharides was sufficient to mediate this effect in tumor-bearing mice [16]. The authors demonstrated the clinical relevance of *Bacteroides* by performing FMT from melanoma patients receiving anti-CTLA-4 immunotherapy to tumor-bearing germ-free mice. FMT from patients that experienced an outgrowth of *Bacteroides* during treatment conferred enhanced tumor control to recipient mice. The authors also discovered protective roles for *Burkholderia* and *B. fragilis* together in mice, which limited immunotherapy-induced intestinal epithelial cell death [16]. Other bacterial species protect patients from the toxic side effects of chemotherapy [73]. These findings uncover a tantalizing new mechanism regulating the delicate balance between efficacy and toxicity, and reveal opportunities for interventions aimed at improving patient outcomes during cancer immunotherapy.

Paradoxically, *B. fragilis* has also been associated with the development of cancer through toxin production [74, 75], and induction of regulatory T cells [76, 77]. Inflammatory products derived from other microbes can also exacerbate malignancy, but the precise mechanisms by which commensals interfere with cancer immunotherapy have yet to be identified [72, 78, 79]. One possibility is modulation of the WNT/ β -catenin pathway. Success of PD-1/PD-L1 blockade for treatment of melanoma depends in part on established T cell infiltration into tumors, which is disrupted by tumor-intrinsic WNT/ β -catenin signaling [80]. Commensal gut bacteria can modulate WNT/ β -catenin pathway and promote colon carcinogenesis [81, 82]. It is unknown if gut microbiota could extend such an influence to tumors outside the gastrointestinal tract, but it is conceivable that commensals at other sites (skin) could engage similar mechanisms to activate WNT/ β -catenin in melanomas. That being said, there is evidence that bacteria in the gut can influence immunity in distant tissues. In the two immunotherapy studies discussed above, the success of checkpoint blockade against subcutaneous tumors in mice was dependent on specific bacterial species in the gut [15, 16]. Another recent study demonstrated how the intestinal microbiota influences allergic inflammation in the lungs [83]. These findings highlight the obvious need to understand how specific commensals influence tumor immunology, and the extent to which gut bacteria affect immunity to cancers in different tissues of the body. The identity of microbial species within individual patients is now easily accessible through advances in ribosomal RNA sequencing. It is tempting to speculate that specific bacteria could soon serve as predictive biomarkers for treatment outcomes or as targets for therapeutic manipulation prior to immunotherapy.

Studying microbiota in mouse models

There are three main approaches to model how microbiota influence biological processes: mice from different facilities of origin, antibiotic treatment, and germ-free (GF) mice. Mice acquired from different facilities are colonized by distinct microbiota dictated by bacteria present in their respective housing locations [15]. These differences model the natural variation among patients, and have a surprisingly powerful influence on antitumor immunity. The provocative message from these studies is that development of new immune modulatory treatments in mice should be confirmed in animals with distinct microbiota. The results further suggest that sequencing the microbiota of patients participating in immunotherapy clinical trials could provide valuable correlates for safety and efficacy.

Mice can be treated with a broad-spectrum cocktail of antibiotics to eliminate many bacterial subtypes. GF mice are completely free of any microbial colonization, but require specialized housing facilities. It is also important to consider that GF mice have impaired immunological development, ranging from decreased recruitment of intraepithelial lymphocytes to altered formation of Peyer's patches [84, 85]. GF and antibiotic-treated models are useful to address gross alterations in immune responses. They can also be deliberately reconstituted with specific microbes of interest by FMT from other experimental animals or patients to study the impact of individual commensals. It is likely within these types of experiments that new microbes with the capacity to influence tumor immunity will be elucidated.

Concluding remarks

The potential of immunotherapy to save the lives of cancer patients is undeniable. The most dramatic and durable responses have come from releasing the regulatory breaks of the immune system through blockade of the inhibitory checkpoint receptors PD-1 and CTLA-4. But responses have been varied, with some patients deriving little benefit and others experiencing severe treatment-related adverse events. Focus has now shifted toward the mechanisms for these disparate responses. What determines the success of immunotherapy among diverse patient populations? Insight has come from recent mouse studies revealing that age, obesity, and microbiota profoundly influence both natural immunity to cancer and the ability to effectively respond to immunotherapy. This area of investigation is in its infancy, but results are sufficiently compelling to force new thinking into how human cancer immunotherapy is modeled in mice. Whereas the bulk of preclinical research has used young healthy mice, we propose that a broader approach is necessary for future therapeutic breakthroughs (see Outstanding Questions). Only through systematic testing in mice that are young and old, lean and obese, and different in microbiota can we hope to recapitulate the complexities of human cancer immunotherapy.

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Outstanding Questions

- Are older cancer patients at greater risk of adverse events during checkpoint blockade immunotherapy due to natural increases in adipose tissue and resulting inflammation?
- Does obesity influence the therapeutic benefit or potential toxicity of checkpoint blockade in patients?
- What commensal species exert a cooperative influence on cancer immunotherapy, and which ones confer protection from treatment-related toxicities?
- How can identification of helpful commensal bacteria lead to their use as probiotics to enhance efficacy and reduce toxicity during cancer immunotherapy?
- Can diversification of mouse models that interrogate the roles of age, obesity and microbiota produce translational discoveries that improve outcomes for cancer patients?

Trends box

- Mouse models of cancer immunotherapy have failed to recapitulate the variable responses and potential toxicity seen in clinical settings.
- Variable outcomes in many patients treated with immunotherapy may be influenced by advanced age and obesity, which are associated with chronic low-level inflammation due to increased adiposity.
- The microbiota has a broad influence on antitumor immunity, and distinct commensals can have cooperative or antagonistic effects on immunotherapy.
- Extending preclinical immunotherapy studies to include young and old mice, lean and obese mice, and mice with diverse microbiota is needed to more accurately model patient populations.

Figure 1. The challenges of immunotherapy in obese mice

(A) Obesity was induced in BALB/c mice fed high fat chow (60% kcal) for 20 weeks. Renal tumor cells were established in the kidney for 7 days followed by treatment with Ad-TRAIL and CpG. Normal weight mice responded to treatment through activation of DCs and induction of IFN γ ⁺ CD8⁺ T cells and arrested tumor growth. Tumors in obese mice receiving the same treatments were infiltrated with regulatory DCs and reduced T cell numbers and progressed rapidly. No toxicity was reported in treated obese mice [13]. (B) In a second study, obesity was induced in C57BL/6 mice either by high fat diet (60% kcal) or genetic ablation of the gene encoding leptin (*ob/ob*). In young normal weight mice with renal cell tumors, immunotherapy with anti-CD40 and IL-2 provided a long-term survival benefit, reliant on DCs and CD8⁺ T cells [86]. The same immunotherapy in obese mice resulted in rapid and lethal toxicity mediated by TNF produced by elevated numbers of macrophages [14]. Immunotherapy with checkpoint blockade antibodies has not yet been tested in obese mice.

Figure 2. The effects of commensal microbiota on cancer immunotherapy

Distinct commensal microbes are known to have a range of effects on host immunity and tumor progression, and even cancer immunotherapy. Bacteria are grouped here according to their potential influences on cancer immunotherapy, based on published reports. This includes evidence that *B. fragilis* and *Burkholderia* combine to protect healthy tissue from therapy-related assault, and that *Bifidobacterium* and *B. fragilis* cooperate with checkpoint blockade to promote antitumor immunity. The identification of cooperative and protective genera and species provide an intriguing possibility of probiotic therapy that could be combined with cancer immunotherapy to improve patient outcomes and even convert non-responders.

Table 1
Key nutritional components of common mouse diets

Normal rodent chow (D12492), NASH chow (D09100301), and high fat chow (D12492) from Research Diets Inc.

	Normal	NASH	High fat
Protein (kcal%)	20	20	20
Carbs (kcal%)	70	40	20
Fat (kcal%)	10	45	60
Sucrose (kcal) Fructose (kcal)	275 0	384 800	275 0
Fructose (kcal)	0	800	0