An observational study of concomitant immunotherapies and denosumab in patients with advanced melanoma or lung cancer

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**ABSTRACT**
After a case report of profound clinical response in a melanoma patient following treatment with an immune checkpoint inhibitor (ICI) and RANK-ligand inhibitor denosumab, we identified similar patients from electronic health records (EHR) and described patient characteristics and outcomes. This 2017 observational study used Flatiron Health’s EHR database from ~255 US cancer clinics. Included were advanced melanoma or non-small-cell lung cancer (NSCLC) patients who received denosumab within 30 days of CTLA-4 (ipilimumab) or PD1 (pembrolizumab, nivolumab) inhibitors start with a minimum of 6 months of follow up. Real-world tumor response (rwTR) was analyzed for scans available up to 30 days after concomitant therapy. Preclinical experiments evaluated sequencing of ICI, denosumab vs monotherapy or control.

Melanoma (n = 66) patients received concomitant denosumab/ICI for a mean 4.0 months, 3.1 months for NSCLC (n = 241). Two-thirds of patients had best rwTR evaluable (complete CR, partial response PR, stable disease SD, or disease progression PD). Longer mean duration of concomitant exposure was associated with overall response rate (ORR; CR+PR) in melanoma (p = 0.0172), NSCLC (p < .0001), and combined cohorts (p < .0001). The disease control rate (ORR plus SD) was 56% amongst melanoma patients and 58% amongst NSCLC patients. Longer concomitant therapy was associated with increased overall survival, primarily in NSCLC (p < .0001). Preclinical data suggest that ICI initiated before or at same time as denosumab was optimal. Results provide proof-of-concept that rwTR is associated with concomitant denosumab/ICI. Crude survival analyses supported the association of concomitant therapy and improved outcomes outside of clinical trials and warrant comparative study.

**Introduction**
Immune system recognition and suppression of malignant cell growth is not a new concept, but is drawing great interest in oncology drug development. In November 2017, approximately 650 studies in Clinicaltrials.gov were enrolling patients for trials targeting the programmed death-1 (PD-1) pathway or one of its ligands (PD-L1), compared with 215 such studies listed in November 2015. Clinical trials of monoclonal antibodies (mAbs) targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4) and PD-L1 have produced remarkable and durable clinical responses leading to a paradigm shift in the treatment of advanced-stage cancers, and to regulatory approvals of these agents for the treatment of several malignancies. Immune-checkpoint inhibitors (ICI) CTLA-4 and PD-1 are T-cell surface receptors that produce immune inhibition at discrete steps in a T-cell response that, if unregulated, can damage the host. These inhibitory processes are up-regulated in many tumors and mAbs reactive with CTLA-4 and PD-1, which block distinct immune-inhibitory receptors on activated T cells, amplify anti-tumor immunity.

Novel immunotherapy-based combinations, particularly those that impinge on non-redundant immunosuppressive mechanisms, may improve clinical responses in advanced melanoma. For example, in October 2014 Smyth et al. reported a 39-year-old woman with metastatic melanoma (BRAF wildtype) who experienced a profound response after concurrent treatment with a CTLA-4 reactive mAb (ipilimumab) and the receptor-activator of nuclear kappaB ligand (RANKL) — specific mAb, denosumab. Another case report demonstrated complete response (CR) in a patient with metastatic melanoma using this combination. And, a recent study in Germany reported that six of ten cases treated with ipilimumab/denosumab had CR or partial response (PR), two with a long-lasting stabilization and...
two with progression of their disease. Furthermore, preclinical data has demonstrated a combination effect of RANKL blockade with anti-CTLA-4, anti-PD-(L)1 mAbs in melanoma, prostate cancer and colon adenocarcinoma. Mechanistically, the addition of a RANKL-specific mAb to ICI mAbs increased T-cell effector function and increased CD8+ T-cell infiltration, leading to increased anti-tumor immunity. Denosumab is routinely prescribed for advanced solid tumors patients with bone metastases or with hypercalcemia, therefore some patients may already receive denosumab in combination with an immunotherapy agent with no reported safety concerns. The potential additive benefit of using RANKL inhibition in combination with PD-(L)1 or CTLA-4 blockade, however, has not been formally assessed in clinical trials.

Here, we provide real-world evidence extending beyond published case studies examining multiple ICI (ipilimumab, nivolumab, pembrolizumab) in two tumor types (advanced melanoma, metastatic non-small cell lung cancer [NSCLC]), and measuring patient outcomes. Specific objectives were: (1) to describe patient demographic, clinical, and treatment characteristics; and (2) to measure clinical response and overall survival in these patient cohorts. In addition, the combined effectiveness and sequence of RANKL blockade and PD-1 inhibition was tested in a preclinical lung cancer model. Together these data describe in greater detail how patients may benefit from ICI immunotherapy combined with denosumab. This work should be considered hypothesis generating and may be used to inform the design of future observational and randomized human studies with comparator arms to more formally evaluate the potential for using electronic health records data to evaluate real-world treatment response and to more definitively quantify the comparative effectiveness of treatment with concomitant ICI and denosumab versus ICI alone.

Results
Concomitant exposure of denosumab and CTLA-4 (ipilimumab) and/or PD-1 (nivolumab, pembrolizumab) inhibitors identified 66 patients diagnosed with advanced malignant melanoma and 241 individuals with metastatic NSCLC (Supplemental Figure 1). Mean age among melanoma patients was 64.6 years, 34.8% were female, and 42 (63.6%) received ipilimumab, 22 (33.3%) nivolumab, and 13 (19.7%) pembrolizumab (9 [13.6%] received ipilimumab/nivolumab; 2 [3.0%] ipilimumab/pembrolizumab combination) concomitantly with denosumab – 71.2% as part of first-line therapy (Table 1). Among NSCLC, the mean age was 68.3 years, 48.1% female, 63.1% non-squamous histology, one patient received ipilimumab, 235 (98%) received nivolumab and 6 pembrolizumab – 21.6% as first-line therapy for advanced disease (Table 1). Over 80% of NSCLC patients had previously received chemotherapy (Supplementary Tables S1A, S1B). Nine percent of melanoma patients had bone only metastases, whereas 30.3% of NSCLC patients had metastatic disease limited to the bone. Eighty-eight percent of NSCLC patients had a history of smoking, and only 14.1% were tested for PD-L1 expression – 54.3% among those tested were deemed positive for PD-L1 expression (Table 1B).

Best response populations (i.e., patients with a response assessment no later than 30 days after the final dose of either concomitant therapy) included 44 (66.7%) melanoma and 166 NSCLC (66.9%) patients, with the following results in these populations. The mean age of patients included in the best response populations was similar to that of excluded patients, although males comprised a slightly larger proportion of excluded patients in both melanoma and NSCLC cohorts. Time since diagnosis was similar for included and excluded patients. Compared with included patients, excluded melanoma patients were less likely to have advanced (Stage 3 or 4) disease at diagnosis, while excluded NSCLC patients were more likely to have advanced disease at diagnosis compared to their included counterparts primarily due to a larger proportion of patients with Stage 3 disease. In the two disease cohorts, the proportions of patients with both bone and visceral metastases was greater among patients excluded from the best response populations than among the included patients. The mean duration (standard deviation [SD]) of concomitant exposure was 161.0 (178.3) days for melanoma patients who were included in the best response population versus 33.6 (21.5) days for excluded patients. Among NSCLC patients in the best response population, the mean duration of concomitant exposure was 126.0 (97.6) days compared with 25.8 (27.8) days in the excluded population.

In the best response populations, the mean number (SD) of imaging assessments was 3.4 (2.6) for melanoma and 2.5 (1.6) for NSCLC patients. Seventy-three percent (32 of 44) of (best response) melanoma patients received anti-PD1 mAb and the remainder received anti-CTLA4 mAb concomitantly with denosumab (there were no instances of anti-PD1 combined with anti-CTLA4 mAbs). The median (interquartile range) follow-up for best response among melanoma patients was 6.59 (2.36;12.1) months, and 7 (16%) had 50 weeks or more of concomitant exposure (5 [71%] with PR/CR) (Figure 1A). Among 24 (55%) melanoma patients with 24 weeks or more of concomitant exposure, 13 (54%) had CR/PR. The overall response rate (ORR; CR plus PR) was 41% in all melanoma patients, 47% among those treated with anti-PD1, and 25% with anti-CTLA4 (Table 2). The disease control rate (DCR; ORR plus SD) was 56% among melanoma patients treated with anti-PD1. In NSCLC, all 166 best response patients had received anti-PD1 mAb concomitantly with denosumab, associated with an ORR of 33% and DCR of 58% (Table 2). Fourteen (8%) patients provided more than 50 weeks of concomitant exposure (9 [64%] with PR/CR) (Figure 1B). Among 69 (42%) NSCLC patients with 24 weeks or more of concomitant exposure, 42 (61%) had CR/PR.

A statistically significant association was observed between concomitant therapy duration and response for melanoma (p = 0.0172), NSCLC (p < .0001) and combined cohorts (p < .0001) (ANOVA). For NSCLC and combined cohorts, better response was associated with longer duration of concomitant therapy with discrete tertile duration categories (p < .0001); statistical significance level was lost in the analysis of melanoma patients as the cell sizes were considerably reduced (p = 0.0621) (Mantel-Haenszel Chi-square). The association was consistent in
analyses of first-line only; majority of best responses were observed during first- or second-line with follow up. Survival analyses using Kaplan-Meier methods and tertile categories for duration of concomitant therapy indicated increased overall survival (OS) following completion of concomitant therapy with longer duration of concomitant use primarily in the NSCLC cohort (p < .0001). Any association of treatment duration with survival was not as clear in melanoma patients due to smaller sample size of each tertile (Figure 2A and B). In an exploratory combined cohort analysis, categories of response show that CR/PR best response was associated with longer survival (log-rank; p < .0001) (Figure 2C).

In the preclinical setting, we tested the anti-tumor efficacy of these combinations in the mouse 3LL lung adenocarcinoma model given the observations in NSCLC patients receiving denosumab and ICI. These experiments were designed to complement the observational research, and to address the optimal sequence of mAb therapy. We compared the tumor growth suppressive activity of concurrent mAb therapy with equivalent total dose of mAbs given as sequential therapy. Preclinical data show combination anti-RANKL and anti-PD-1 mAbs were superior in efficacy to either monotherapy or control Ig, irrespective of whether therapies were given concurrently or sequentially (Figure 3). Sequencing anti-PD-1 therapy prior to anti-RANKL treatment led to a significantly greater reduction in tumor volume as compared with anti-RANKL treatment prior to anti-PD-1 therapy (p < 0.01) (Figure 3).

Table 1. Demographic characteristics of advanced melanoma and NSCLC cohorts (response data set).

|                  | Melanoma                      | NSCLC                       |
|------------------|-------------------------------|-----------------------------|
|                  | Included (N = 44)             | Excluded (N = 22)           | Included (N = 166) | N = 75 |
| Age at index date (years)*, Mean (SD) | 63.0 (14.0)         | 67.8 (13.2)          | 68.9 (10.0)       | 67.0 (9.3) |
| Sex, n (%)       | Female 17 (38.6)              | 6 (27.3)               | 83 (50.0)         | 33 (44.0) |
|                  | Male 27 (61.4)                | 16 (72.7)              | 83 (50.0)         | 42 (56.0) |
| Race/Ethnicity, n (%) | White/Caucasian 31 (70.5) | 15 (68.2)             | 113 (68.1)        | 49 (65.3) |
|                  | Other 2 (4.6)                 | 0 (0.0)                | 25 (15.0)         | 12 (16.0) |
|                  | Unknown 11 (25.0)             | 7 (31.8)               | 28 (16.9)         | 14 (18.7) |
| Time (years) from diagnosis to index, mean (SD) | 2.7 (2.5)           | 3.0 (3.7)              | 1.2 (1.3)         | 1.2 (1.3) |
| Highest ECOG, n (%) | 0 – 1 15 (34.1)              | 5 (22.7)               | 51 (30.7)         | 26 (34.7) |
|                  | ≥ 2 11 (25.0)                 | 4 (18.2)               | 86 (51.8)         | 27 (36.0) |
|                  | Unknown 18 (40.9)             | 13 (59.1)              | 29 (17.5)         | 22 (29.3) |
| Stage at diagnosis, n (%) | I 4 (9.1)           | 6 (27.3)               | 12 (7.2)          | 4 (5.3) |
|                  | II 9 (20.5)                  | 4 (18.2)               | 9 (5.4)           | 1 (1.3) |
|                  | III 9 (20.5)                 | 3 (13.6)               | 13 (7.8)          | 13 (17.3) |
|                  | IV 13 (29.5)                 | 5 (22.7)               | 126 (75.9)        | 55 (73.3) |
|                  | Unknown 9 (20.5)             | 4 (18.2)               | 6 (3.6)           | 2 (2.7) |
| Metastatic disease, n (%) | Bone only 5 (11.4)          | 1 (4.5)                | 52 (31.3)         | 21 (28.0) |
|                  | Multiple 39 (88.6)            | 21 (95.5)              | 114 (68.7)        | 54 (72.0) |
| Histology, N (%) | Squamous cell NA             | NA                    | 57 (34.3)         | 21 (28.0) |
|                  | Nonsquamous cell              |                       | 105 (63.3)        | 47 (62.7) |
|                  | Unknown                      |                       | 4 (2.4)           | 7 (9.3) |
| Pre-index systemic chemotherapy, n (%) | 2 (4.5) | 1 (4.5) | 140 (84.3) | 61 (81.3) |
| PD-L1 tested, n (%) | 0 (0) | 1 (4.5) | 26 (15.7) | 9 (12.0) |
| PD-L1 status among those tested**: PD-L1 positive, n (%) | 0 (0) | 1 (4.5) | 16 (9.6) | 4 (5.3) |
| Immuno- oncology agent, n (%) | Nivolumab 17 (38.6) | 5 (22.7) | 163 (98.2) | 72 (96.0) |
|                  | Pembrolizumab 10 (22.7)      | 3 (13.6)               | 3 (1.8)           | 3 (4.0) |
|                  | Iplimimumab 25 (56.9)        | 17 (77.2)              | 1 (0.6)           | 0 (0.0) |
| First line of therapy with concomitant use^, n (%) | 1st 31 (70.5) | 16 (72.7) | 37 (22.3) | 15 (20.0) |
|                  | 2nd 13 (29.5)                | 6 (27.3)               | 129 (77.7)        | 60 (80.0) |
| Duration (days) of concomitant use, mean (SD) | 161.0 (178.3) | 33.6 (21.5) | 126.0 (97.6) | 25.8 (27.8) |
| Number of assessments (scans), mean (SD) | 3.4 (2.6) | – | 2.5 (1.6) | – |

*Index date is the first date of concomitant use of an immunotherapy + denosumab, as identified based on the structured medication administration for the immunotherapy
** Biomarker status percentages are out of those patients who were tested for that particular biomarker. There are some instances where a patient had multiple tests for a particular biomarker. In such cases, the most recent successful test is displayed in this table.
^ Concomitant use is defined as the administration of denosumab at any point prior to and no later than 30 days following initiation of immunotherapy.

Table 2. Clinical response evaluated among melanoma and NSCLC patients treated concomitantly with denosumab and immune checkpoint inhibitors.

|                  | Melanoma | NSCLC |
|------------------|----------|-------|
|                  | PD1 n (%)   | CTLA-4 n (%) | All Patients n (%) | PD1 n (%) |
| Total            | 32 (100) | 12 (100) | 44 (100) | 166 (100) |
| Complete/Partial response | 15 (46.9) | 3 (25.0) | 18 (40.9) | 55 (33.1) |
| Stable disease   | 3 (9.4)  | 3 (6.8)  | 42 (25.3) |  |
| Progressive      | 14 (43.8) | 9 (75.0) | 23 (52.3) | 69 (41.6) |
Discussion

Building on published case studies of melanoma patients with remarkable clinical responses when treated with anti-RANKL and anti-CTLA-4 mAbs in combination, we examined response in a larger series of patients with either advanced melanoma or metastatic NSCLC treated with concomitant ICI and denosumab. We observed that PR/CR were associated with longer duration of concomitant use of immunotherapy and denosumab. Crude survival analyses supported associations between concomitant therapy duration and improved response, as well as association of PR/CR with survival. Recognizing the limitations of this single-arm, descriptive, observational study, these results provide encouraging proof-of-concept that warrant further investigations.

Skeletal metastases are generally considered a negative prognostic factor in solid organ malignancies. In NSCLC and melanoma, bone metastases and/or skeletal-related events (SREs) are associated with lower overall survival. Taking these factors into account, the clinical responses observed in this real-world study of patients with skeletal metastases compare favorably with outcome measures (ORR and DCR) reported in pivotal trials of ICI (Table 3A, 3B). Most strikingly, the ORR in advanced NSCLC patients treated concurrently with denosumab and anti-PD1 mAb was 33.1%. Other notable characteristics of the study cohort are a preponderance of non-squamous histology (typical of NSCLC), and a relatively high level of prior exposure to chemotherapy (>80%). Published response rates in advanced NSCLC (not selected by PD-L1 expression) to anti-PD1 mAb monotherapy...
range from 18–20% for second line treatment to 23–24.8% for first-line treatment (Table 3A). Importantly, although tumor PD-L1 expression was infrequently assessed in this real-world cohort, the proportion staining positive was comparable to that recorded in these clinical trials; where patients were selected on the basis of PD-L1 positivity (CheckMate 026, KEYNOTE-024), response rates were strikingly higher (Table 3A).

The ORR among melanoma patients treated with concurrent anti-PD1 mAb (mostly first line) and denosumab in our real-world study was 46.9%, which is encouraging when compared with ORR reported in trials utilizing anti-PD1 mAb first-line monotherapy or immunotherapy-naive patients, which range from 33.7% to 45% (Table 3B). In contrast, among melanoma patients in our study who received

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**Figure 2.** A Overall survival for advanced melanoma cohort measured after completion of concomitant treatment duration (tertiles) of denosumab and immune checkpoint inhibitors (full analysis set). B Overall survival for advanced NSCLC cohort measured after completion of concomitant treatment duration (tertiles) of denosumab and immune checkpoint inhibitors (full analysis set). C Overall survival for advanced melanoma and NSCLC cohorts measured after completion of combined by best response category achieved with concomitant denosumab and immune checkpoint inhibitors (best response set).
ipilimumab concurrently with denosumab, 25% achieved ORR, while published phase 2–3 clinical trials for first line ipilimumab in melanoma reported ORR in the range of 11–19%. 4,22,23

An important caveat when interpreting these rwTR data is the use of response criteria in the EHR dataset, whereby a pragmatic real-world modification of RECIST was applied in a time-point fashion. This is likely less stringent in comparison with response assessment used in prospective clinical trials; on the other hand, assessment intervals for response in routine care were likely less structured and perhaps more infrequent than in the trial setting. A practical consequence of this is that some patients designated to have achieved PR in the EHR cohort may have achieved SD per RECIST. Reassuringly, when an alternative composite outcome measure was compared (DCR, which includes the category of SD in addition to responders), the EHR cohort similarly compared favorably to the published prospective trials (Table 3A,B).

Real-world studies can play a key role in identifying predictors of response, nonresponse and resistance, so that therapies are prescribed for the right patient (maximizing benefit), and avoiding the wrong patient that is unlikely to benefit from therapy (minimizing risk). These studies also have the potential to inform novel drug targets, validate efficacy (effectiveness) of a medicinal product, and possibly reduce the costs and timelines associated with drug development. With the 21st Century Cures Act, the US Food and Drug Administration (FDA) has recognized the value of real-world evidence and is developing a regulatory framework to use these data to support new indications for approved drugs or to satisfy post-approval study requirements.

While prospective clinical trial results provide informative context for observational real-world studies which analyze data obtained in the routine care setting, it is necessary to consider critical differences between these two types of research. Clinical trial populations are selected using stringent selection criteria that exclude many patients in the community who are represented in real world studies, thereby dampening magnitudes of effect with respect to clinical response and survival outcomes. This phenomenon is described in the epidemiology literature and confirmed by a recently published analysis of pembrolizumab-treated melanoma patients from community oncology clinics, 24 where authors specifically noted that characteristics often used as exclusion from clinical trials – brain metastases, elevated LDH, poor performance status (ECOG PS>1), and third-line or later PD-1 therapy – were associated with worse patient outcomes. Therapeutic exposures also differ, as indicated by the fact that 70% of patients in that particular study discontinued pembrolizumab during the study period. A key strength of real-world evidence is the ability to complement clinical trial data by providing insights into the performance of therapies in a more diverse (older ages, greater disease progression/burden) patient population. In fact, a recent study 25 comparing characteristics of patients with NSCLC treated with PD-1 inhibitors in clinical trial cohorts to those in the real-world during the first year after US regulatory approval found that real-world patients were older at treatment initiation and were more likely to have had smoking history relative to clinical trial cohorts.

Therefore, when comparing real-world to clinical trial data, these population differences could result in bias – known as confounding by indication – such that treatments, particularly newly approved therapies, may look less effective and less safe than described in clinical trials because they are given to sicker individuals from the "real world". Despite this potential bias – and recognizing that physicians do not record treatment responses in EHR consistent with RECIST as well as other limitations around timing, quality, and availability of tumor response assessments 26 – the observed tumor response rates in our study compare favorably to those reported in clinical trials. We note that while it is possible that real world response is underestimated our results compare well with single institution reports of real world non-concomitant use of anti-PD1 for NSCLC (Supplementary Table S2A). 27–30

In addition, although the number of patients with advanced
Table 3A. Comparison of objective response rates from anti-PD-1 pivotal clinical trials in NSCLC.

| Trial          | Current EHR Study (Liede et al) | CheckMate 057 | CheckMate 017 | CheckMate 012 | CheckMate 026 | KEYNOTE-001 | KEYNOTE-024 |
|---------------|---------------------------------|---------------|---------------|---------------|---------------|-------------|-------------|
| Phase         |                                  |               |               |               |               |             |             |
| Anti-PD1      | Nivolumab and pembrolizumab      | 3             | 3             | 18            | 3             | 18          | 3           |
| Histology     | Non-squamous and squamous        | 1st and 2nd   | 2nd           | 1st           | 1st           | 1st         | 1st         |
| Treatment line (advanced disease) | 2nd                 |               |               |               |               |             |             |
| Number of subjects (n) | 166                 | 287*          | 131*          | 52            | 208           | 495         | 154**       |
| ORR (%)       | 33                              | 19            | 20            | 23            | 26*           | 24.8 (1st line) | 44.8        |
| DCR (%)       | 58.4                            | 44            | 49            | 50            | 64*           | 51.5**      | N.R.***     |
| PD-L1 positive of assessable (%) | 54                             | 53            | 47            | 70            | 100^^^        | 60.8*       | 100**       |

* Number of subjects in nivolumab arm
^ Primary efficacy analysis population (PD-L1 expression ≥ 5%); nivolumab arm
** Number of subjects in pembrolizumab arm
^^ Includes patients with "non-complete response/non progressive disease" who did not have measurable disease per RECIST
*** N.R. (not reported)
^^^ PD-L1 expression ≥ 5% in primary efficacy population
# PD-L1 positive tumor expression in screened population
## Inclusion criteria: tumoral PD-L1 expression > 50%
melanoma in this study who received ipilimumab concurrently with denosumab is limited, the ORR is markedly higher than those real-world outcomes reported with ipilimumab alone (Supplementary Table S2B).31–36

The precise cellular source of RANKL necessary for antitumor activity of RANKL blockade may include infiltrating lymphocytes, lymph nodes or tumor cells themselves, and further analysis of potential mechanisms behind the observed favorable rwTR are warranted.37 In NSCLC, a population where an exploratory post-hoc analysis of phase 3 clinical trial data reported improved survival with denosumab,38 RANKL is observed not only in infiltrating lymphocytes, but also in tumor epithelium of 75% of adenocarcinoma samples,39 and high RANKL levels are adversely associated with survival.40 Detailed analysis of RANK and RANKL expression in melanoma has not been described.41 While the anti-tumor efficacy observed in preclinical models was enhanced upon combination of RANKL blockade and anti-PD-1 antibody irrespective of sequence, pre-treatment with anti-PD-1 prior to anti-RANKL was superior to other sequences. We have observed that anti-PD-1 increased RANKL expression on activated T-cells in a colon carcinoma model,15 suggesting that RANKL/RANK may act as a negative feedback mechanism after checkpoint inhibition. Thus, the specific sequence of denosumab administration versus ICI may improve tumor response and should be tested in clinical trials and preclinical mechanistic studies. Altogether, these findings now prompt an examination of the impact of ICI on RANKL/RANK expression and function in the tumor microenvironment in melanoma and lung cancer.

The findings of our study were intended to provide proof-of-concept, and future research should focus on: (1) a comparative effectiveness of ICI mAbs with or without concomitant denosumab (120 mg); (2) further analysis of the optimal use (combinations, duration, and sequencing) of ICI; and (3) identifying patient characteristics or biomarkers that may predict responders and guide treatment assignments.

This real-world study of substantial cohorts of metastatic melanoma and NSCLC patients treated concurrently with denosumab and immunotherapies indicates clinical responses that compare favorably with randomized clinical trials and smaller retrospective, real-world studies of ICI given as monotherapies. These results build on several case studies to suggest that RANKL blockade may enhance the activity of ICI thus, leading to improved tumor control in patients, similar to what has been described in preclinical mechanistic studies. Moreover, 42% to 55% of patients in the best response cohorts were treated concurrently with denosumab and immunotherapy for 24 weeks or longer, demonstrating extended periods of use. These data prompt further studies to verify the potential improvement of tumor response by the addition of RANKL blockade to ICI and translational studies to determine the complementary mechanisms leading to enhanced anti-tumor immunity.

**Patients and methods**

**Study design**

This is a retrospective observational cohort study of patients with a diagnosis of advanced melanoma or metastatic NSCLC who received concomitant treatment with anti-RANKL mAb (denosumab, 120 mg) plus CTLA-4 (ipilimumab) and/or PD-1 (pembrolizumab, nivolumab) inhibitory mAbs.

**Data source**

Data for this study were obtained from Flatiron Health’s longitudinal, demographically and geographically diverse database derived from patient-level electronic health records (EHR), agnostic to EHR system. At the time of this project, the database drew from ~255 cancer clinics from across the US (1.7 million patients), representing mainly community-based oncology practices. Records include diagnostic and treatment details captured during routine clinical care, either as structured data (diagnostic details, laboratory values, prescribed drugs) or unstructured data (scanned reports, physician notes). Patient-level data were processed and harmonized centrally by Flatiron Health, and stored in a secure, HIPAA-compliant manner. Unstructured data processing utilized technology-enabled abstraction, described elsewhere.42 Institutional Review Board approval of the study protocol was obtained prior to study conduct and informed patient consent was waived. Data provided for the study were de-identified with provisions in place to prevent re-identification to protect patients’ confidentiality.

**Patients**

Two distinct cohorts were selected: advanced melanoma or metastatic NSCLC (Supplemental Figure 1). Inclusion criteria from structured data were: confirmed diagnosis via ICD codes (melanoma ICD-9 172.x or ICD-10 C43.x or D03.x; and NSCLC ICD-9 162.x or ICD-10 C34.x or C39.9), two visits

| Trial | Current EHR study (Liede et al.) | CheckMate 066 | CheckMate 067 | KEYNOTE-001 | KEYNOTE-006 |
|-------|---------------------------------|---------------|---------------|-------------|-------------|
| Phase | Anti-PD1 mAb | Nivolumab or pembrolizumab | Nivolumab | Nivolumab | Pembrolizumab | Pembrolizumab |
| Treatment line | ORR (%) | DCR (%) | ORR (%) | DCR (%) | ORR (%) | DCR (%) |
| 1st | 32 | 46.9 | 316 | 43.7 | 133 | 45 ** |
| 2nd | 210 | 40.0 | 51 | 54.5 | 279 ± 277 ^ ^ |
| 3rd | 56.3 | 56.7 | 51 | 51 | 33.7–32.9 |

* Immunotherapy naive

^ Nivolumab arm

** Subset of treatment-naïve patients (1st line treatment only)

^^ Pembrolizumab arms, various doses

*** Includes patients with “non-complete response/non progressive disease” who did not have measurable disease per RECIST

**Table 3B. Comparison of anti-PD1 pivotal clinical trials in melanoma.**

This is a retrospective observational cohort study of patients with a diagnosis of advanced melanoma or metastatic NSCLC who received concomitant treatment with anti-RANKL mAb (denosumab, 120 mg) plus CTLA-4 (ipilimumab) and/or PD-1 (pembrolizumab, nivolumab) inhibitory mAbs.
on or after January 1, 2011, and co-administration of immunotherapy (ipilimumab, nivolumab, or pembrolizumab) and denosumab. Concomitant therapy was defined as receipt of denosumab at any point prior to ICI initiation, or no later than 30 days following ICI initiated at least 6 months before the data cutoff (November 30, 2016), and no later than second line of therapy. Clinical abstractors confirmed cutaneous melanoma or NSCLC (and advanced disease), defined as stage III or IV melanoma or stage IIIB or IV NSCLC at initial diagnosis, or recurrent disease following an earlier stage diagnosis.

"Real-world" endpoints

Tumor response was extracted from documents in the EHR from routine clinical care. This "real world" tumor response (rwTR) variable was generated by trained abstractors using a predefined process to identify imaging assessments, review associated clinical documentation, and report the treating clinician’s interpretation of the imaging. RECIST criteria were adapted for real-world application, with each imaging assessment mapped to one of the following categories: CR, PR, stable disease (SD), progressive disease (PD), pseudoprogression, indeterminate response, and not documented. In contrast to RECIST criteria, PR was defined as any reduction in visible disease on imaging short of a CR.

Mortality data was generated by supplementing structured EHR death data with unstructured EHR and external commercial data. The quality of this derived mortality data has been assessed for a large cohort by measuring sensitivity, specificity, positive and negative predictive values, and date agreement, against the highest-completeness US mortality data, the National Death Index (NDI); sensitivity ranged from 76–83% and specificity ranged from 96–99% (unpublished data).

Preclinical examination of anti-pd1 and anti-RANKL

C57BL/6 wild-type (WT) mice were bred-in-house at the Queensland Institute of Medical Research Berghofer (QIMRB). All experiments were approved by the QIMRB Animal Ethics Committee. The mouse lung carcinoma cell line 3LL (kindly provided by Dr. Robert Wiltrout, NCI Frederick, MD), was injected, maintained and monitored as previously described. Purified anti-mouse anti-RANKL (IK22-5; rat IgG2a) and control mAbs (1–1, rat IgG2a) were purchased from BioXcell (West Lebanon, NH). Anti-PD1 (RMP1-14, rat IgG2a) was purchased from Leinco (St Louis, MO). Digital calipers were used to measure the perpendicular diameters of the tumors. The tumor size from Leinco (St Louis, MO). Digital calipers were used to measure the perpendicular diameters of the tumors. The tumor size for tumors was calculated as the product of the two measurements and is presented as mean ± SEM.

Analysis

Descriptive analyses are provided. Significance testing was limited to comparisons between discrete groups using one-way ANOVA for continuous variables, or Chi-square test for binary variables and Mantel-Haenszel Chi-Square for multiple categories. Analyses performed on rwTR data used response assessments no later than 30 days after the final dose of either concomitant therapy. These resulting data were analyzed to derive the mean number of imaging assessments and best response. Overall response rate was calculated based on the number of patients with either a CR or PR as their best response. Survival analyses were based on Kaplan-Meier methods with log-rank for significance testing. For comparison of treatment groups in preclinical sequencing experiment, one-way ANOVA was performed, with Tukey’s post-test for multiple comparisons.

Disclosures of interest

A. Liede and R.K. Hernandez are employees and stockholders of Amgen Inc. S. Wade is employed by Wade Outcomes Research and Consulting which has received remuneration from Amgen Inc. R. Bo has no financial interests to disclose. N.C. Nussbaum is an employee and stockholder of Flatiron Health, which is an independent subsidiary of the Roche Group. E. Ahern has received a speaker’s honorarium from Amgen Australia and travel accommodations/expenses from MSD Australia. W.C. Dougall has received a speaker’s honorarium from Amgen and research funding from Bristol Myers Squibb. M.J. Smyth has research agreements with and has consulted to Bristol Myers Squibb, Aduro Biotech, Corvus Pharmaceuticals, and Tizona Therapeutics, consulted to Arcus Biosciences, provided expert testimony to Corvus Pharmaceuticals, and holds patents or intellectual property rights with QIMR Berghofer and Bristol Myers Squibb.

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