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Tumor Microenvironment Remodeling Following Neoadjuvant Immunotherapy in Solid Tumors

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Abstract

Neoadjuvant immune checkpoint blockade, delivered before rather than after surgical resection of a solid tumor, has moved in roughly a decade from a mechanistic hypothesis into a standard of care across non-small cell lung cancer, triple-negative breast cancer, urothelial carcinoma, and melanoma. This article synthesizes the clinical and mechanistic literature on how a short neoadjuvant course of checkpoint blockade remodels the tumor immune microenvironment, drawing on landmark randomized trials (CheckMate 816, KEYNOTE-671, KEYNOTE-522, NEOSTAR, ABACUS, and the OpACIN-neo/OpACIN program) that establish the magnitude of pathologic and survival benefit, together with the single-cell and spatial biology literature that has begun to resolve what that remodeling consists of at cellular resolution: reinvigoration and clonal expansion of pre-existing CD8 T cells, a shift in the effector-to-regulatory T cell balance, partial repolarization of tumor-associated macrophages, and a supportive role for tertiary lymphoid structures. We give particular weight to the paired pre- and post-treatment biopsy studies that this treatment sequence uniquely enables, while noting that the mechanistic literature remains concentrated in a small number of tumor types and modest cohort sizes relative to the large trial populations establishing clinical efficacy. Read together, the evidence supports neoadjuvant checkpoint blockade as a reproducible driver of favorable tumor immune microenvironment remodeling across multiple solid tumor types, while leaving predictive biomarker validation, myeloid- and stroma-directed mechanistic detail, and generalization beyond lung cancer and melanoma as an open research agenda.

Keywords: tumor microenvironment; neoadjuvant immunotherapy; immune checkpoint blockade; CD8 T cell exhaustion; tertiary lymphoid structures

1. Introduction

Neoadjuvant immunotherapy — giving immune checkpoint blockade before, rather than after, surgical resection — has moved from a scientifically interesting idea to a standard of care across several solid tumor types in roughly a decade. The logic for treating before surgery rather than after is not primarily about tumor shrinkage, although that happens too; it is about immunology. A tumor left in place during treatment retains its full complement of tumor antigens, its draining lymphatics, and its resident and infiltrating immune populations, so a checkpoint inhibitor given in the neoadjuvant window has an intact battlefield on which to reactivate a systemic anti-tumor T cell response, one that can in principle continue to patrol for micrometastatic disease long after the primary tumor is removed [1,2]. Removing the tumor first and treating afterward, by contrast, removes much of that antigen depot before the immune system has had a chance to be properly re-educated by it. This is not a new theoretical insight — tumor-draining lymph nodes and the primary tumor bed have long been understood as privileged sites of T cell priming — but it took the maturation of checkpoint blockade as a clinical tool, and a decade of trials across lung cancer, melanoma, breast cancer, and urothelial carcinoma, to turn the argument into a body of practice-changing evidence.

What distinguishes neoadjuvant immunotherapy from adjuvant or metastatic-setting immunotherapy, from a research standpoint, is that it hands investigators something they almost never get: a paired, on-treatment biopsy of the whole tumor at the time of definitive surgery. A patient who receives checkpoint blockade in the metastatic setting is followed by imaging and, occasionally, a needle biopsy of a single lesion; a patient who receives neoadjuvant checkpoint blockade before a planned resection has their entire treated tumor, together with locoregional lymph nodes, removed and examined under the microscope on a fixed schedule, typically some four to nine weeks after the first dose. That resected specimen is where "tumor microenvironment remodeling following neoadjuvant immunotherapy" stops being a hypothesis and becomes a directly observable phenomenon: pathologists and, increasingly, single-cell and spatial biologists can compare treated tumors against a reference set of matched untreated tumors and describe, with a level of granularity that metastatic-setting biopsies rarely allow, exactly which immune and stromal populations expanded, contracted, or changed state in response to treatment, and how those changes track with pathologic response and eventual relapse-free survival [3,4].

This article synthesizes that literature. We are not reporting new experimental results; we are trying to give a coherent account of what the tumor immune microenvironment (TIME) looks like before checkpoint blockade, what changes have now been measured across multiple tumor types and multiple independent research groups after a short course of neoadjuvant treatment, what those changes appear to predict about outcome, and where the mechanistic and clinical open questions remain. We organize the discussion around the biology of the pretreatment tumor microenvironment as a target for immunotherapy; the mechanisms by which checkpoint blockade is thought to remodel that microenvironment; the accumulated clinical trial evidence for neoadjuvant immune checkpoint blockade across lung, breast, melanoma, and urothelial cancer, which both establishes the clinical importance of the question and supplies the response categories (pathologic complete response, major pathologic response) that the mechanistic literature is trying to explain; the single-cell and spatial studies that have begun to resolve what "remodeling" actually consists of at the level of individual cells and their spatial neighborhoods; the specific contributions of myeloid cells, cancer-associated fibroblasts, and tertiary lymphoid structures, each of which behaves differently from the CD8 T cell compartment that dominates most mechanistic narratives; and, finally, the resistance mechanisms and unresolved questions that the field has been candid about even as the clinical results have accumulated.

It is worth stating the scope plainly at the outset. Most of the single-cell and spatial mechanistic literature synthesized here comes from a handful of tumor types — principally non-small cell lung cancer (NSCLC) and melanoma, with growing contributions from urothelial carcinoma and triple-negative breast cancer — and from cohorts that are, almost without exception, modest in size: tens rather than hundreds of patients, because paired pre- and post-treatment tissue with adequate quality for single-cell or spatial assays is genuinely hard to collect. The clinical trial evidence for neoadjuvant checkpoint blockade's efficacy is, by contrast, drawn

from large randomized trials with thousands of enrolled patients across multiple tumor types. Holding those two evidence bases side by side — a strong, large-cohort clinical case that neoadjuvant immunotherapy improves outcomes, alongside a smaller but increasingly consistent set of mechanistic studies describing how the tumor microenvironment actually changes under that treatment — is the organizing structure of this review.

2. The Tumor Immune Microenvironment as a Therapeutic Target

Before describing what changes under treatment, it is necessary to describe what is being changed. The tumor immune microenvironment is not a passive backdrop to malignant cell proliferation; avoiding immune destruction and enabling a tumor-permissive local immune state are counted among the core hallmarks of cancer in the most recent update of that framework, alongside the genomic and proliferative hallmarks that have anchored cancer biology since the concept was first proposed [5]. A solid tumor is, in this framing, best understood as an ecosystem: malignant epithelial or mesenchymal cells, a vascular and lymphatic network, cancer-associated fibroblasts and other stromal cells, and an immune infiltrate that spans essentially every lineage of the innate and adaptive immune system, all interacting in a spatially organized and often quite stereotyped architecture that differs systematically between tumor types and, within a tumor type, between patients [4].

Binnewies and colleagues' influential framework for understanding the TIME organizes this ecosystem around the question of what is functionally limiting anti-tumor immunity in a given tumor: whether T cells are essentially absent from the tumor bed (an immune "cold" or excluded phenotype, in which the vasculature or stroma may be physically preventing infiltration); whether T cells are present but functionally suppressed, for instance by myeloid-derived suppressive populations or by chronic antigen exposure driving exhaustion; or whether antigen presentation itself is defective, such that even a fully competent T cell has nothing correctly displayed to recognize [4]. Galon and Bruni's complementary "hot, altered, and cold" taxonomy captures much the same idea in more clinically oriented language and, usefully, links each category to a distinct combination-therapy rationale: hot tumors, already T cell infiltrated, are the ones where checkpoint blockade alone is most likely to work; cold and altered tumors need something else, priming, trafficking, or myeloid reprogramming, layered on top of checkpoint blockade before a T cell response can be unleashed at all [6]. This taxonomy matters directly for neoadjuvant immunotherapy because pretreatment biopsies routinely show that a meaningful fraction of any given solid tumor cohort — commonly a third to half of patients, depending on tumor type — starts out in the cold or altered category, and those patients are disproportionately represented among the non-responders in every neoadjuvant trial reviewed in this article.

The T cell compartment specifically has received the most sustained mechanistic attention, in part because PD-1/PD-L1 and CTLA-4 blockade act directly on T cell biology and in part because CD8 T cell density and location within the tumor are, empirically, among the strongest available correlates of response to checkpoint blockade across tumor types [7]. Tumeh and colleagues' foundational analysis of pre- and on-treatment melanoma biopsies from patients receiving PD-1 blockade showed that CD8 T cells were already present at the invasive tumor margin in most patients before treatment began, but were prevented from infiltrating the tumor core by a chronic, PD-L1-mediated adaptive resistance program; blocking PD-1 released that brake and allowed pre-existing, tumor-reactive T cells already positioned at the margin to move inward and proliferate, rather than recruiting an entirely new T cell repertoire from scratch [7]. That finding, more than any single subsequent study, established the conceptual model that most of the mechanistic literature on TME remodeling still operates within: checkpoint blockade in a responsive tumor does not create anti-tumor immunity out of nothing, it reinvigorates and redistributes an immune response that was already, in some latent or geographically constrained form, present.

Not every tumor cooperates with this model, however, and understanding why is central to the resistance literature discussed later in this article. Sharma and colleagues' widely cited taxonomy divides checkpoint-therapy failure into primary resistance (the tumor never had the T cell infiltrate, antigenicity, or antigen-presentation machinery needed to respond in the first place), adaptive resistance (an initial productive immune response is actively suppressed by the tumor,

for instance via PD-L1 upregulation or myeloid recruitment, in a way checkpoint blockade can potentially reverse), and acquired resistance (a tumor responds initially and then relapses, often through loss of antigen presentation or interferon-signaling components under the selective pressure of the very immune response the treatment induced) [8]. All three categories are directly relevant to the neoadjuvant setting, where a single, time-limited course of treatment offers a uniquely clean window to observe which mechanism is operating in a given patient, because the resected specimen captures the tumor's state at a fixed, short interval after treatment began rather than after months or years of ongoing selective pressure.

3. Mechanisms of Immune Remodeling Under Checkpoint Blockade

The mechanistic account that has emerged from paired pre- and post-treatment sampling studies over the past decade converges on a small number of recurring, tumor-type-spanning themes, even though the specific magnitude and kinetics vary considerably by tumor type and by which checkpoint (PD-1/PD-L1 versus CTLA-4, or both together) is being blocked.

The first and most consistently reported change is expansion and phenotypic shift of the CD8 T cell compartment. Riaz and colleagues' longitudinal analysis of melanoma patients treated with nivolumab, which sequenced tumors at baseline and again during treatment, found that responding tumors showed expansion of pre-existing intratumoral T cell clones — the same reinvigoration-of-an-existing-repertoire pattern Tumeh and colleagues had described — together with a broadening of the T cell receptor repertoire that was more pronounced in tumors with lower baseline mutational burden, suggesting that clonal expansion and repertoire diversification are, to some degree, compensatory routes to an effective response rather than a single fixed mechanism [3]. Within that expanding CD8 compartment, exhaustion state turns out to matter as much as raw cell number. Miller and colleagues showed that exhausted CD8 T cells are not a uniform population but stratify into a less-differentiated, TCF7-expressing progenitor-exhausted subset that retains proliferative capacity and a terminally exhausted subset that does not, and that the therapeutic benefit of checkpoint blockade tracks specifically with the progenitor-exhausted population's capacity to proliferate and partially differentiate upon PD-1 blockade, rather than with reversal of exhaustion in the terminal subset [9]. This distinction is one of the more clinically actionable pieces of mechanistic biology to come out of the TME-remodeling literature, because it reframes what a post-treatment biopsy showing an expanded, activated CD8 infiltrate is actually measuring: expansion of a specific progenitor subset, not uniform reinvigoration of every exhausted T cell present at baseline.

The second recurring theme is that regulatory T cell (Treg) dynamics move in the opposite direction from effector CD8 T cells in most, though not all, responding tumors, and that this divergence is itself informative. A shift in the ratio of cytotoxic effector cells to Tregs, rather than either population's absolute count in isolation, has repeatedly emerged as more discriminating between responders and non-responders than CD8 density alone, consistent with the broader TIME framework's emphasis on the balance of stimulatory and suppressive signals rather than any single cell population viewed independently [4]. CTLA-4 blockade, which acts partly by depleting intratumoral Tregs via antibody-dependent mechanisms in addition to its T cell co-stimulatory effects, appears to shift this balance more forcefully than PD-1/PD-L1 blockade alone — an effect first established clinically in the metastatic-melanoma setting, where combined nivolumab plus ipilimumab produced higher response rates than either agent alone at the cost of substantially increased immune-related toxicity [10] — which is one plausible mechanistic contributor to the higher pathologic response rates seen with combination nivolumab-plus-ipilimumab regimens relative to nivolumab monotherapy in the neoadjuvant NSCLC trials discussed in the next section [11].

The third theme, and arguably the one with the least settled mechanistic account, concerns antigen presentation and the tumor cell compartment itself. Cristescu and colleagues' pan-tumor analysis of genomic and transcriptomic correlates of PD-1 blockade response across multiple KEYNOTE trials found that tumor mutational burden and a T cell-inflamed gene expression signature were independently predictive of response, and that tumors combining high mutational burden with a pre-existing inflamed phenotype had the highest response rates of any subgroup examined, while tumors low in both had the lowest — an observation

consistent with the idea that both antigenicity (enough mutated, potentially immunogenic protein to present) and a pre-existing capacity to mount an inflammatory response are independently necessary, and that checkpoint blockade's remodeling effect on the TME is most robust when it has both ingredients to work with rather than either alone [12]. This helps explain why response rates differ so markedly across tumor types with different average mutational burdens — melanoma and NSCLC, both smoking- or UV-mutagenesis-driven cancers with comparatively high mutational burden, are overrepresented among the tumor types where neoadjuvant checkpoint blockade has shown the largest effect sizes, a pattern borne out directly in the trial evidence reviewed next.

4. Clinical Evidence from Neoadjuvant Immunotherapy Trials

The mechanistic literature described above would be an interesting academic exercise on its own; what makes it clinically consequential is that a series of randomized and single-arm phase 2 trials, across several tumor types, has now shown that these microenvironment changes translate into measurable pathologic and, in the most mature trials, survival benefit.

In resectable non-small cell lung cancer, CheckMate 816 randomized patients to three cycles of platinum-doublet chemotherapy with or without nivolumab before surgery, and found a pathologic complete response rate — no viable tumor cells identified in the resected primary tumor or sampled lymph nodes — of 24.0% with the addition of nivolumab, compared with 2.2% for chemotherapy alone, an approximately eleven-fold relative increase that also translated into improved event-free survival at longer follow-up [13]. KEYNOTE-671 tested a closely related perioperative design — pembrolizumab or placebo added to neoadjuvant chemotherapy, followed by adjuvant pembrolizumab or placebo after surgery — in a similar resectable NSCLC population, and reported a two-year event-free survival of 62.4% with the pembrolizumab-containing regimen versus 40.6% with chemotherapy alone, establishing that the pathologic response signal seen with CheckMate 816 corresponds to a durable, clinically meaningful survival advantage rather than a purely histologic curiosity [14]. The NEOSTAR trial, a smaller randomized phase 2 study in the same resectable NSCLC population, directly compared neoadjuvant nivolumab monotherapy against combined nivolumab plus a single dose of ipilimumab, and found a major pathologic response rate (10% or less viable tumor) of 22% with nivolumab alone versus 38% with the combination, consistent with the mechanistic expectation, described above, that adding CTLA-4 blockade produces a more forceful shift in the T cell effector-to-Treg balance than PD-1/PD-L1 blockade alone [11].

Table 1: Pathologic and survival outcomes reported in five landmark neoadjuvant/perioperative immune checkpoint blockade trials spanning four solid tumor types.

Trial (Tumor Type)	Source	Regimen Compared	Reported Result
CheckMate 816 (resectable NSCLC)	Forde et al. [13]	Neoadjuvant nivolumab + chemotherapy vs chemotherapy alone	Pathologic complete response 24.0% vs 2.2% (43/179 vs 4/179)
KEYNOTE-671 (resectable NSCLC)	Wakelee et al. [14]	Perioperative pembrolizumab + chemotherapy vs placebo + chemotherapy	2-year event-free survival 62.4% vs 40.6%
KEYNOTE-522 (early triple-negative breast cancer)	Schmid et al. [15]	Neoadjuvant pembrolizumab + chemotherapy vs placebo + chemotherapy	Pathologic complete response 64.8% vs 51.2%
NEOSTAR (operable NSCLC)	Cascone et al. [11]	Neoadjuvant nivolumab alone vs nivolumab + ipilimumab	Major pathologic response 22% vs 38%
ABACUS (muscle-invasive urothelial carcinoma)	Powles et al. [16]	Neoadjuvant atezolizumab monotherapy (single arm)	Pathologic complete response 29% (20/68)

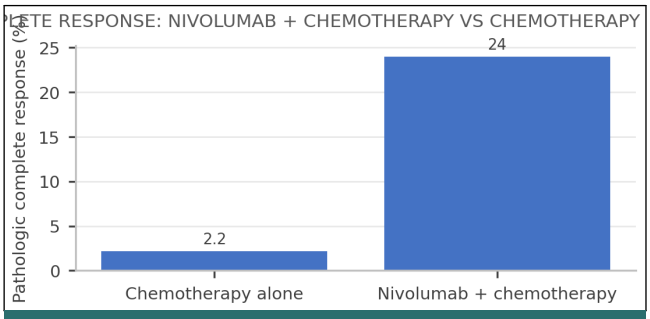

Figure 1: Pathologic complete response rate with the addition of neoadjuvant nivolumab to platinum-doublet chemotherapy versus chemotherapy alone in resectable non-small cell lung cancer, as reported in the CheckMate 816 randomized phase 3 trial [13].

The same pattern — a substantial, statistically robust increase in pathologic response with neoadjuvant checkpoint blockade added to standard-of-care therapy — recurs outside lung cancer. In early-stage triple-negative breast cancer, KEYNOTE-522 randomized patients to neoadjuvant chemotherapy with or without pembrolizumab, followed by adjuvant

pembrolizumab or placebo, and reported a pathologic complete response rate of 64.8% in the pembrolizumab arm versus 51.2% with chemotherapy alone [15]. In muscle-invasive urothelial (bladder) carcinoma, the single-arm ABACUS trial tested neoadjuvant atezolizumab monotherapy, without concurrent chemotherapy, ahead of cystectomy, and reported a pathologic complete response rate of 29% (20 of 68 evaluable patients) [16] — a notable result in part because it demonstrates a meaningful pathologic response to checkpoint blockade given entirely without cytotoxic chemotherapy, in a tumor type where cisplatin-based chemotherapy has historically been considered the only effective neoadjuvant option. In stage III melanoma, the OpACIN-neo trial systematically compared three different dosing and scheduling combinations of neoadjuvant ipilimumab plus nivolumab specifically to identify a schedule that preserved efficacy while reducing the substantial immune-related toxicity of the full-dose combination, and follow-up survival and biomarker analyses from the same research group linked pathologic response after neoadjuvant combination checkpoint blockade to markedly better relapse-free survival than was seen in non-responding patients, reinforcing that pathologic response in the resected specimen is not merely a convenient surrogate endpoint but tracks with the clinical outcome that actually matters to patients [17,18]. A separate melanoma study found that meaningful pathologic and molecular changes could be detected after a single dose of neoadjuvant PD-1 blockade, well before the multi-week treatment courses used in most of the trials above, and that the magnitude of that single-dose response predicted subsequent relapse-free survival — evidence that at least the earliest phase of TME remodeling can occur, and be clinically informative, on a timescale of days rather than weeks [19].

Table 1 summarizes the pathologic and survival endpoints from five of these trials side by side. Two features of the table are worth drawing out explicitly. First, the absolute magnitude of benefit varies considerably by tumor type and regimen — the roughly eleven-fold relative increase in pathologic complete response seen in CheckMate 816 is larger than the more modest, though still statistically robust, absolute difference seen in KEYNOTE-522 — and some of that variation is plausibly explained by the mutational-burden and baseline-inflammation arguments discussed in the previous section. Second, every trial in the table used a slightly different pathologic response definition (complete response, defined as no viable tumor, versus major pathologic response, typically defined as 10% or less viable tumor), a heterogeneity in endpoint definition that complicates direct cross-trial and cross-tumor-type comparison and that the field has only partially standardized.

5. Single-Cell and Spatial Insights into Microenvironment Remodeling

Bulk pathologic response rates, however clinically important, describe an outcome without resolving the underlying cellular process. The past several years have seen a wave of single-cell RNA sequencing and spatial profiling studies applied specifically to paired pre- and post-neoadjuvant-immunotherapy tumor tissue, aiming to describe TME remodeling with cellular, rather than bulk-tissue, resolution.

The most directly relevant of these studies to this article's title profiled non-small cell lung cancer tumors before and after neoadjuvant immunotherapy using single-cell RNA sequencing across roughly ninety thousand cells drawn from three pretreatment and twelve post-treatment tumors, comparing patients who achieved a major pathologic response against those who did not [20]. The results substantially extend, at single-cell resolution, the population-level picture described in the previous two sections. Cytotoxic CD8 T cells and CD16-positive natural killer cells both expanded and shifted toward more activated transcriptional states after treatment, while regulatory T cells contracted — directly consistent with the effector-to-Treg rebalancing theme discussed above — and these shifts, particularly expansion of effector-memory (GZMK-high) and tissue-resident-memory (GZMB-high) CD8 subsets, were more pronounced in patients who went on to achieve a major pathologic response than in those who did not [20]. The same study reported that tumor-associated macrophages shifted toward a more "neutral" transcriptional phenotype after treatment, moving away from the pro-tumor, immunosuppressive program myeloid cells commonly adopt in untreated tumors, though notably without fully converting to a classically pro-inflammatory, anti-tumor phenotype — a nuance that complicates any simple narrative in which

checkpoint blockade straightforwardly "reprograms" tumor-associated macrophages from foe to friend. Among rarer populations, an aged, CCL3-expressing neutrophil subset was specifically depleted in patients with major pathologic response, and a memory B cell subset defined by co-expression of FCRL4 and FCRL5, together with a CX3CR1-positive monocyte subset, were both enriched in responders and proposed as candidate predictive biomarkers for future validation [20].

These single-cell findings connect to, and help mechanistically ground, the CD8 exhaustion-subset biology described earlier: an expansion specifically of effector-memory and tissue-resident-memory CD8 populations after treatment, rather than uniform expansion of every T cell subset present, is consistent with Miller and colleagues' argument that the therapeutically relevant reservoir under checkpoint blockade is a specific, proliferation-competent progenitor-exhausted or memory-like subset rather than the exhausted T cell pool as a whole [9]. It is also consistent with Riaz and colleagues' melanoma finding that responding tumors show expansion of pre-existing clones rather than wholesale replacement of the T cell repertoire [3] — the NSCLC single-cell data essentially provide a phenotypic, transcriptome-resolved picture of what that clonal expansion looks like from the inside.

Spatial context adds a further dimension that transcriptomic profiling of dissociated single cells, by design, cannot capture: which cells are physically adjacent to which other cells, and how that neighborhood structure changes with treatment. This matters because a T cell's functional state depends substantially on what it is physically touching — an activated dendritic cell, a suppressive macrophage, a fibroblast barrier — and not only on which genes it transcribes in isolation. The broader tertiary lymphoid structure literature, discussed in the next section, is in large part a story about spatial organization: functional, germinal-center-containing lymphoid aggregates within or adjacent to the tumor are a spatial phenomenon that dissociated single-cell sequencing can suggest (via the presence of naive and memory B cell and follicular helper T cell transcriptional signatures) but cannot directly visualize, which is why spatial transcriptomics and multiplexed imaging have become increasingly standard complements to single-cell RNA sequencing in the most recent wave of TME-remodeling studies [21].

6. Myeloid Cells, Stroma, and Tertiary Lymphoid Structures

The mechanistic account so far has centered on T cells, for the reasonable historical reason that checkpoint blockade acts directly on T cell receptors and that most of the earliest and highest-powered mechanistic studies were designed around T cell readouts. But an increasingly influential strand of the TME-remodeling literature argues that myeloid cells, stromal fibroblasts, and organized lymphoid aggregates are not simply downstream bystanders of the T cell response but active, sometimes rate-limiting, participants in whether that response can occur at all.

Tumor-associated macrophages are the largest myeloid population in most solid tumors and are, in their more common tumor-promoting polarization state, a well-established source of the immunosuppressive cytokine and metabolic milieu that keeps T cells exhausted and excluded in the first place. Mantovani and colleagues' review of macrophages as both tools and targets in cancer therapy lays out the mechanistic case that tumor-associated macrophages contribute to checkpoint-blockade resistance through several parallel routes — direct suppression of T cell function via arginase and other metabolic mechanisms, physical exclusion of T cells from the tumor parenchyma, and, in some contexts, direct clearance of antibody-opsonized tumor cells that can blunt the efficacy of certain combination regimens — and that therapeutic strategies aimed at repolarizing or depleting these cells are being developed specifically to complement, rather than replace, T cell-directed checkpoint blockade [22]. The NSCLC single-cell finding described in the previous section, that tumor-associated macrophages shift toward a more neutral but not fully anti-tumor phenotype after neoadjuvant immunotherapy, fits within this framework as a partial, incomplete version of the repolarization that combination myeloid-targeted strategies are trying to complete more forcefully [20].

Tertiary lymphoid structures — ectopic, organized lymphoid aggregates that form within or at the margin of tumors and can contain functional germinal centers, follicular dendritic cells, and the full complement of B and T cell zones normally found in a lymph node — have emerged over the past several years as one of the more robust histologic correlates of checkpoint blockade response across tumor types, and their presence or

induction is increasingly discussed as a spatial signature of favorable TME remodeling rather than an incidental finding. Sautès-Fridman and colleagues' review traces the accumulated evidence that tertiary lymphoid structure density and maturity correlate with improved response to immune checkpoint blockade across melanoma, lung, and several other solid tumor types, and proposes that these structures function as local sites of in situ T cell priming and B cell affinity maturation, effectively supplying the tumor microenvironment with an on-site antigen-presentation and memory-generation capacity that would otherwise require trafficking to a distant lymph node [21]. This framework offers a plausible explanation for why some tumors mount a durable, systemically protective response to a short course of neoadjuvant checkpoint blockade while others show transient pathologic response without lasting immunologic memory: the presence of a functional tertiary lymphoid structure may be what converts a local burst of T cell reinvigoration into a self-sustaining, memory-generating immune response capable of controlling micrometastatic disease long after the primary tumor is resected.

Taken together, the myeloid and stromal literature complicates the picture painted by pure T cell-centric narratives of TME remodeling in a useful way: pathologic response after neoadjuvant checkpoint blockade appears to require not just an expandable T cell compartment but a permissive myeloid and stromal context — an already-present or inducible tertiary lymphoid structure, a macrophage population capable of shifting even partially away from its suppressive baseline state — and the tumors that fail to respond are, in many documented cases, failing along one of these non-T cell axes rather than because they simply lack tumor-reactive T cells altogether [4,22].

7. Resistance Mechanisms and Open Challenges

Several gaps and unresolved questions recur across the literature synthesized here, and it is worth naming them plainly rather than treating the clinical and mechanistic successes described above as the whole story.

First, endpoint heterogeneity and cross-trial comparability remain a genuine problem. As Table 1 illustrates, pathologic complete response, major pathologic response (typically 10% or less viable tumor), and event-free survival are all used, sometimes within the same trial program, as primary or key secondary endpoints, and the relationship between short-term pathologic response and long-term survival benefit, while directionally consistent across every trial reviewed here, has not been formally established as a validated surrogate relationship for every tumor type and regimen combination discussed [23]. Second, resistance biology in the neoadjuvant setting is comparatively understudied relative to the metastatic setting, where longer treatment courses and more accessible on-treatment biopsies have produced a richer picture of acquired resistance mechanisms such as interferon-signaling pathway loss or beta-2-microglobulin mutation; because neoadjuvant treatment is deliberately short and followed promptly by surgery, the acquired-resistance category in Sharma and colleagues' taxonomy is, almost by design, harder to observe directly in this setting than primary or adaptive resistance are [8]. Third, the myeloid and stromal remodeling literature described in the previous section remains considerably less mature, in terms of cohort size and mechanistic specificity, than the T cell literature, and Hegde and Chen's widely cited enumeration of outstanding challenges in cancer immunotherapy specifically flags the difficulty of therapeutically targeting the myeloid compartment, and of resolving spatial heterogeneity within a single tumor, as two of the field's most significant unsolved problems, both of which bear directly on how confidently the field can currently interpret myeloid-lineage findings from neoadjuvant TME-remodeling studies [24].

Fourth, and perhaps most consequentially for translating this literature into clinical decision-making, predictive biomarkers that reliably identify, before treatment begins, which patients will achieve pathologic response remain elusive. Tumor mutational burden and a T cell-inflamed gene expression signature are the most extensively validated pretreatment correlates available, and even in combination they leave substantial unexplained variance in response across the pan-tumor cohorts in which they were characterized [12]. The candidate biomarkers emerging from the single-cell literature described above — the FCRL4/FCRL5 memory B cell signature and CX3CR1-positive monocyte signature identified in the NSCLC cohort discussed in this review, for instance — are, by the reporting group's own framing, hypothesis-generating findings from a modest single-cohort study rather than validated, clinic-ready biomarkers, and require confirmation in

independent, adequately powered cohorts before they can inform treatment selection [20]. Fifth, essentially all of the mechanistic single-cell and spatial literature synthesized in this article comes from a small number of tumor types, dominated by NSCLC and melanoma; how well the specific cellular remodeling patterns described here generalize to tumor types with different baseline mutational burden, different resident stromal architecture, or different standard-of-care chemotherapy backbones — pancreatic, ovarian, and hepatocellular cancers being notable examples where neoadjuvant checkpoint blockade has been studied far less extensively — is, honestly, not yet well characterized.

We do not think any of these gaps undermine the central, well-replicated finding that neoadjuvant checkpoint blockade produces a measurable, clinically consequential remodeling of the tumor immune microenvironment across multiple solid tumor types. But readers should treat the specific cellular and molecular narrative offered in this article — CD8 T cell reinvigoration and clonal expansion, a shift in the effector-to-regulatory T cell balance, partial myeloid repolarization, and a supportive role for tertiary lymphoid structures — as the current best synthesis of a still-developing evidence base, not as a settled mechanistic consensus.

8. Conclusion

The evidence reviewed here supports a fairly specific set of claims about what neoadjuvant immunotherapy does to the tumor immune microenvironment in solid tumors, and why that matters clinically. Across resectable lung cancer, early triple-negative breast cancer, muscle-invasive urothelial carcinoma, and stage III melanoma, adding checkpoint blockade to a short, defined neoadjuvant treatment window produces substantially higher rates of pathologic response than standard-of-care therapy alone, and in the trials with sufficiently mature follow-up, that pathologic response translates into meaningfully improved event-free and relapse-free survival [13,14,15,11,16,18]. The mechanistic literature converges on a reasonably coherent, though not yet fully resolved, account of how that clinical benefit arises: pre-existing, tumor-reactive CD8 T cells at or near the tumor margin are released from chronic checkpoint-mediated suppression and expand, with the specific progenitor-exhausted and memory-like subsets driving most of that expansion; the balance between these effector cells and immunosuppressive regulatory T cells shifts favorably, particularly with combined PD-1 and CTLA-4 blockade; tumor-associated macrophages move, at least partially, away from their default suppressive polarization state; and tertiary lymphoid structures, where present or induced, appear to supply the in situ priming and memory-generation capacity that helps convert a local treatment effect into durable, systemic disease control [7,3,9,22,21].

What the field does not yet have is a validated, prospectively actionable way to predict, before the first dose is given, which patients will realize this benefit, nor a fully resolved account of the myeloid, stromal, and spatial biology that the single-cell literature has only recently begun to describe systematically. Closing those gaps will likely require larger, prospectively designed paired-biopsy cohorts spanning a wider range of tumor types than the current NSCLC- and melanoma-dominated literature, deployed alongside, rather than as a substitute for, the pathologic and survival endpoints that the randomized trials reviewed in this article have already established as clinically meaningful. The consistency with which independent trial programs across four distinct tumor types have now converged on the same qualitative result — that a short course of neoadjuvant checkpoint blockade remodels the tumor immune microenvironment in a direction that measurably improves outcomes — is, in our reading, the strongest available argument that this is a durable feature of solid tumor immunology rather than an artifact of any single trial design, and one that deserves continued, tumor-type-broadening mechanistic investigation rather than treatment as an already-closed question.

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Ethical Approval

Not applicable. As a synthesis of previously published literature, this article did not involve new human participants, animal subjects, or primary data collection requiring ethical review.

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