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Basophil Activation Test: Bridging Allergy and Oncology for Diagnostic, Therapeutic and Prognostic Applications in AllergoOncology: An EAACI Position Paper

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Abbreviations: Alpha-Gal (α-Gal), Galactose-α-1,3-galactose; BAT, Basophil activation test; BTR, Breakthrough reactions; CD-sens, Basophil allergen threshold sensitivity; CRS, Cytokine Release Syndrome; CSU, Chronic spontaneous urticaria; DD, Drug desensitization; EAACI, European Academy of Allergy and Clinical Immunology; EC 50, Effective concentration 50; EGFR, Epidermal growth factor receptor; fMLP, N-Formylmethionyl-leucyl-phenylalanine; FPP2, Filtering Face Piece level 2; HSR, Hypersensitivity reaction; HVA, Hymenoptera venom allergy; ICI, Immune checkpoint inhibitor; iDHR, Immediate Drug hypersensitivity reaction; IL, Interleukin; irAEs, Immune-related adverse events; LAD-2, Laboratory of Allergic Diseases (LAD2) mast cell line; LTC4, Leukotriene C4; LTP, Lipid Transfer Protein; MAT, Mast cell activation test; MFI, Mean fluorescence intensity; MRGPRX2, Mas-related G protein coupled receptor X2; PKC, Protein kinase C; PPE, Personal protective equipment; sIgE, Specific IgE; SPT, Skin Prick Test; SSC, Side Scatter; ST, Skin test; SWOT, Strengths, Weaknesses, Opportunities and Threats; TAT, T cell activation test; TEAEs, Treatment-emergent adverse events; Th2, T helper 2; TME, Tumour microenvironment; VIT, Venom immunotherapy.

ABSTRACT

The basophil activation test (BAT) is gaining increasing relevance as an ex vivo functional assay in allergy to evaluate IgE-mediated hypersensitivity reactions to food allergens, venoms, and drugs and to monitor tolerance induction. Establishing universal standard operating protocols has been difficult, due to several challenges including variable activation markers, positive control selection, the need for processing fresh blood samples, and the existence of non-releasing individuals. In oncology, BAT is also an emerging promising diagnostic and management tool to assess hypersensitivity reactions to biologics and chemotherapy agents, monitor drug tolerance in desensitisation, and predict and address the safety of novel anti-cancer IgE-based therapeutics. This position paper highlights the emerging significance of BAT in AllergoOncology, in facilitating therapy monitoring, biomarker discovery, and risk stratification. Capitalising on long-acquired expertise in the development of BAT for allergy, we propose research directions and routes to clinical applications of this highly promising tool in AllergoOncology. We advocate the need for enhanced focus on addressing standardisation challenges and leveraging outputs for precision medicine. By linking allergy and oncology, the key remaining limitations can be addressed, with the aim of realising the significant promise of BAT as a robust tool to enhance personalised care in allergy and AllergoOncology.

1 | Introduction: Basophils, Their Functions and Application of the Basophil Activation Test (BAT) in Allergy

Basophils are rare leukocytes that mature from haematopoietic stem cells in the bone marrow and are mobilised as fully differentiated cells into the peripheral blood, constituting < 1% of total white blood cells in humans. The precise ontology of basophils is not yet completely clear. In humans, a shared basophil/eosinophil progenitor has been described, while distinct basophil/mast cell progenitors have also been reported in animal models [1, 2]. In some ways, basophils resemble mast cells, which are found in tissue, primarily located near blood vessels and mucosal surfaces, whereas basophils are circulating leukocytes. Basophils have numerous cytoplasmic secretory granules filled with proinflammatory mediators and express a wide range of cell surface receptors that facilitate activation through multiple pathways. Among them, fully functional IgE receptors (FcεRI) can bind allergen-specific IgE (sIgE) with high affinity and be cross-linked by multivalent allergen interactions. This results in basophil activation and rapid degranulation, with the release of histamine, tryptase, and other proinflammatory mediators, including certain eicosanoids (e.g., leukotriene C4 (LTC4) that cause the characteristic allergic reactions). In addition to IgE-mediated activation, basophils can be triggered by a wide range of stimuli [3]. These include N-formylmethionine-leucyl-phenylalanine (fMLP), complement anaphylatoxins (C5a or C3a) that activate these cells via a G-protein coupled receptor, and immune complexes of antigens formed with IgG functioning through recognition of Fcγ receptors. Additionally, certain experimental non-physiological conditions induce basophil degranulation in vitro, such as hypo- or hyper-osmolar conditions, direct increase of cytosolic Ca2+ by A23187, or activation of protein kinase C (PKC) by phorbol ester (Figure 1) [3, 4].

Basophils are dynamic cells in the circulation and can be mobilised by chemokine signals that can induce their degranulation, especially in combination with other stimuli such as platelet-activating factor or interleukin (IL)-3. Basophils are also an early source of IL-4 and other type 2-related cytokines. As such, basophils play an important role in allergic diseases, contributing to both the early induction of Th2 responses and to the allergic late phase. This involvement is marked by their selective migration to affected tissues, as observed in asthma, rhinitis and atopic dermatitis [5]. After insect venom- or food-induced anaphylaxis, basophils decrease in the circulation most likely due to activation and migration into tissues [6].

The physiological role of basophils is mainly in the defence against parasites such as helminths, but studies in mouse models also suggest that they play essential roles in the defence against ticks (*Haemaphysalis longicornis*) [7, 8]. Besides this and their role in allergic diseases, basophils are involved in a variety of other pathologies including cancer, myocardial infarction, autoimmunity, fibrosis, chronic obstructive pulmonary disease [9, 10]. Activated basophils have been observed in the blood of patients with chronic urticaria and systemic lupus erythematosus. Additionally, IgE autoantibodies and activated basophils are factors associated with disease activity in lupus nephritis [11, 12]. Basophils in the circulation and within the tumour microenvironment (TME) may also contribute to anti-tumour immunity and patient outcomes [13].

The propensity of basophils to be stimulated by suspected allergens can be tested in BAT, a functional cell-based assay performed in whole human blood ex vivo. BAT mimics the activation of basophils in human circulation when these cells encounter either IgE or non-IgE mediated stimuli. BAT is increasingly becoming an established assay in a growing number of routine diagnostic laboratories in the field of allergy, to predict, diagnose, and monitor hypersensitivity reactions (HSR) to different allergens (i.e., food, hymenoptera venoms, and therapeutic drugs).

In this position paper, we explore and advocate for the potential of the BAT as a valuable tool in the field of AllergoOncology. We review its emerging applications, adapted from allergy to oncology and AllergoOncology, for translational cancer research, studying HSR to cancer therapies, and to aid diagnosis, monitoring, and stratification. We discuss the challenges of implementing BAT in oncology and highlight the importance of and opportunities for collaboration between allergy and oncology experts. We propose key research directions and routes to achieve clinical application of this highly promising allergy tool as a validated and widely used assay for AllergoOncology research and patient benefit.

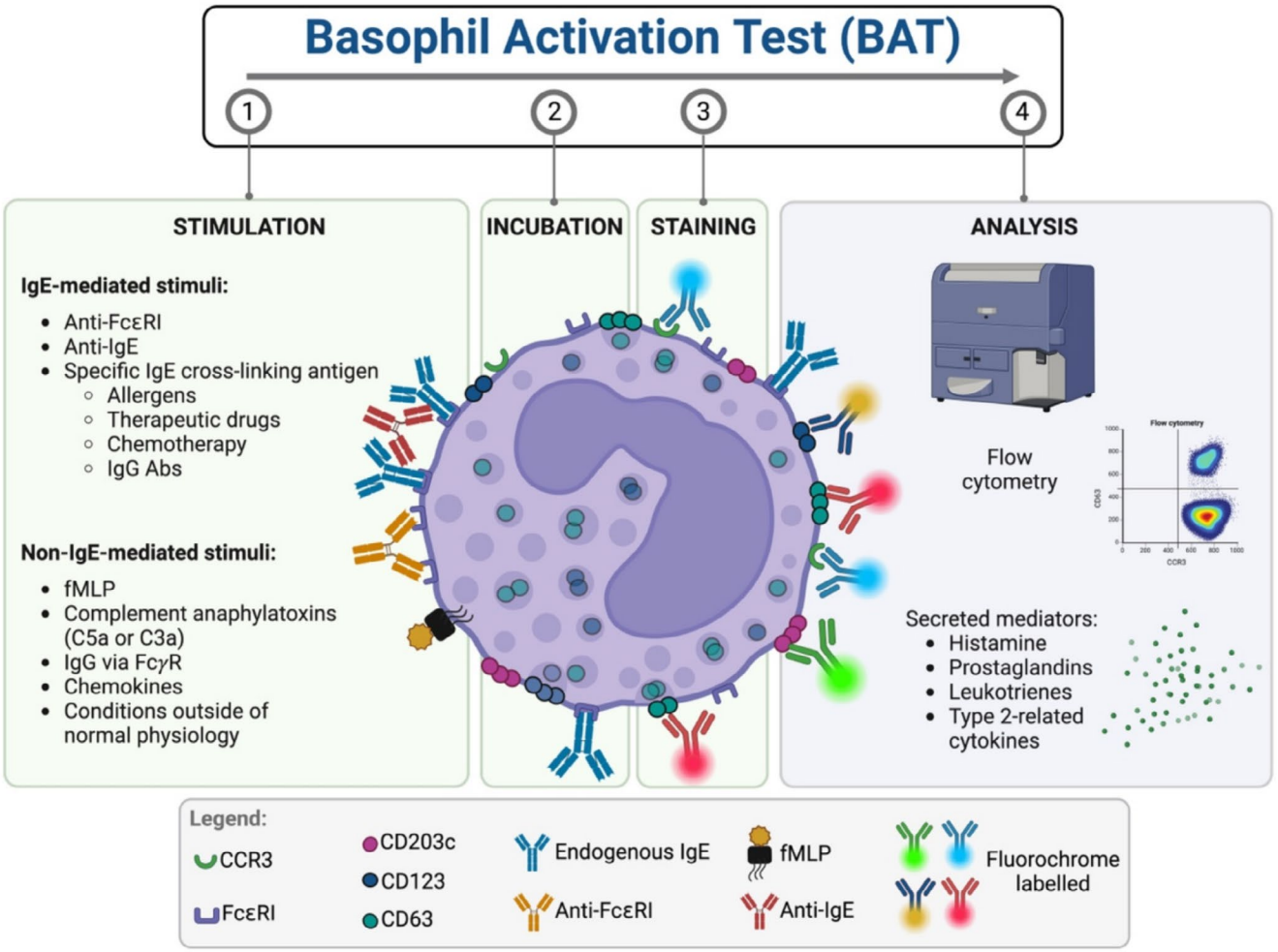

FIGURE 1 | Flow diagram of the process of basophil stimulation and evaluation using BAT. (1) Stimulation: Key examples of IgE-mediated and non-IgE mediated stimuli used in BAT are highlighted. IgE-mediated stimuli activate basophils by cross-linking endogenous IgE (e.g., via polyclonal anti-IgE antibody) or through direct cross-linking of FcεRI using an anti-FcεRI antibody. Non-IgE mediated stimuli, such as fMLP or complement anaphylatoxins, activate basophils via alternative pathways. (2) Incubation: Stimuli are incubated with whole blood ex vivo for usually 20–30 min at 37°C, typically in a water bath or incubator. During activation, basophils upregulate activation markers such as CD63, which translocate from cytoplasmic vesicles to the cell surface. CD203c, another activatory and identification marker, also increases in surface expression upon activation. (3) Staining: Fluorochrome-labelled antibodies are added to identify basophils and quantify the upregulation of surface activation markers. Comparing stimulated samples to unstimulated controls allows assessment of basophil activation. In some protocols, incubation and staining occur simultaneously. (4) Analysis: After incubation, supernatants can be collected to measure secreted mediators. Samples are then treated to lyse red blood cells, centrifuged, and washed, and cells are resuspended in appropriate media. Flow cytometry can be performed immediately or on fixed samples stored at 4°C. Analysis involves measuring percentage of cell expressing or the mean fluorescence intensity (MFI) of activation markers and comparing stimulated samples to unstimulated controls. For allergen testing, patient hypersensitivity is assessed relative to maximal activation by positive controls and baseline values. Created with BioRender.com.

2 | Application of the BAT in Allergy

During basophil activation ex vivo (i.e., BAT) to different stimuli (such as polyclonal anti-IgE, anti-FcεRI antibody, allergen and fMLP), quantifiable bioactive soluble mediators are secreted and generated (such as histamine, tryptase, leukotrienes and cytokines). Furthermore, changes in the expression of basophilic surface membrane markers, such as CD63 and CD203c, also occur and can be detected by flow cytometry using specific monoclonal antibodies (Figure 1) [14, 15]. Due to the minimal expression of Mas-related G-protein coupled receptor member X2 (MRGPRX2) receptor on resting basophils, BAT has been thought not suitable for assessing MRGPRX2-mediated degranulation by certain drugs that are potent MRGPRX2 agonists. It is noteworthy that this receptor can be upregulated in basophils due to preanalytical procedures like cell purification with magnetic beads [16, 17].

The BAT requires the detection of basophils in fresh unfractionated whole blood, commonly identified as low side scatter (SSC), and $CD193^+$, $CD203c^+$ and/or $CD123^+HLA\text{-}DR^-$ cells [18, 19]. The assay involves an ex vivo challenge, typically initiated by IgE-mediated stimuli through the engagement of IgE and allergens, which cross-link the FcεRI receptors. Therefore, relying on FcεRI or IgE labelling for detecting basophils could interfere with allergen-IgE interactions or may even trigger basophil

activation directly. For assessing basophil activation, the plasma membrane expression of the tetraspanin molecule, CD63, normally located on secretory granules, or CD203c, which is constitutively expressed by basophils but upregulated upon activation, can be measured as a signal of activation and a surrogate for degranulation [20, 21]. Basophil reactivity is defined as the number of basophils that respond to a given stimulus, and basophil sensitivity refers to the stimulant concentration required to trigger activation of half of all reactive basophils [22].

A detailed description of the main strenghts and limitations of BAT has very recently been reviewed [19, 23–25]. We briefly highlight that the readouts of the BAT can be influenced by different factors such as selection of activation markers, use of IL-3 within the stimulation buffer (which is known to increase basophil sensitivity to stimuli), nature and concentration of stimuli (whole extract/native drug, components, or metabolites), and patient's current treatment regimen. Moreover, analysis and interpretation of the test outcome: basophil reactivity (as a percentage of cells expressing the activation marker or stimulation index) and basophil sensitivity ('CD-sens': inversion of the concentration at which 50% of basophils respond [EC50]) are also critical [22].

Additionally, factors such as the time elapsed between blood collection and analysis, the duration and condition of blood storage, and the interval between the clinical reaction and test performance are all known to influence the readouts [22, 26–28]. Importantly, BAT cannot be interpreted on subjects displaying 'non-releaser' basophils, which may occur due to spleen tyrosine kinase (Syk) deficiency (about 10%–20% of the population) [29–31] or treatments with corticosteroid drugs [13]. These conditions can change over time, are independent of skin mast cell activation, and the clinical implications remain unknown [22]. False-negative BAT results may occur immediately after an anaphylactic episode [19]. Moreover, recent allergen exposure could influence baseline activation of basophils. Technical issues, predominantly blood handling, can also cause high background activation (> 2%–2.5% CD63+ basophils). It is possible to interpret results as a positive BAT response when the allergen-specific basophil activation shows at least a Stimulation Index (SI) of 2 or above [28]. Standardisation of these aspects is required for BAT to become a widely available tool (Figures 3 and 4).

BAT is becoming a well-established assay in a growing number of routine diagnostic laboratories, applied for the in vitro diagnosis of type I hypersensitivity reactions triggered by food allergens [32], hymenoptera venoms [33] and drugs (i.e., active ingredients, excipients and/or metabolites) [23], as well as in monitoring allergen immunotherapy, natural resolution, and clinical responses to immunomodulatory treatment for food allergies [18, 24, 34–36]. Recently, BAT has been included in the European Academy of Allergy and Clinical Immunology (EAACI) Clinical Guidelines of Food Allergy Diagnosis, not only for its value on diagnosis, but also to predict reactions to food, helping in the discrimination of clinical relevance and prognosis, potentially reducing the need for oral food challenges (e.g., in polysensitized nuts- and seed-allergic children and in shrimp allergy) [24, 37–39]. Nevertheless, BAT implementation in clinical practice depends largely on its availability in the clinical labs.

Altogether, BAT has emerged as a useful tool to aid the detection of allergies to a broad range of allergenic sources, predict the severity of allergic reactions, and monitor responses to therapeutic interventions to treat allergies. Decreased basophil activation that accompanies allergen immunotherapy can be due to intrinsic mechanisms (basophil anergy) or extrinsic processes like the induction of IgA and IgG antibodies that compete with IgE for allergen binding, thus reducing the amount of allergen that can cross-link sIgE on the basophil surface, and therefore the chance of an allergic reaction or its severity. The BAT is also useful in monitoring response to omalizumab, which captures circulating IgE and reduces IgE bound to receptors on effector cells, leading to progressive reduction of FcεRI expression on basophils and thus response to the allergen in the BAT. Nevertheless, omalizumab can paradoxically increase basophil reactivity to the allergen due to a reduction of the surface FcεRI density on effector cells, enhancing their intrinsic sensitivity [18]. Consequently, patients with higher allergen-specific activity (i.e., those with a higher proportion of IgE that is allergen-specific) most likely respond better to omalizumab. Indeed, BAT can potentially be useful in assessing the response to other biologicals in terms of their effect on the risk of acute reactions to a given allergen [18].

2.1 | BAT for Evaluating Allergic Disease Prognosis and Severity

BAT has been used to monitor allergic disease progression or resolution, and response to treatment. Basophil sensitivity ('CD-sens') seems particularly useful in measuring change over time in allergic patients, reflecting the shift in response to different allergen concentrations, with greater sensitivity being associated with response at lower concentrations [40].

There are various examples of BAT as a monitoring, prognosis, and/or response biomarker in different diseases. The BAT has been found useful in confirming the diagnosis of autoimmune urticaria, identifying subtypes of chronic spontaneous urticaria (CSU), assessing response to omalizumab, as well as cyclosporinesponsiveness in this context [18, 41]. BAT identified children who had developed spontaneous tolerance to cow's milk allergy and could have cow's milk reintroduced in the diet [42]. Similarly, BAT was found useful prior to evaluating the need of an oral food challenge to evaluate tolerance of baked goods [24, 43, 44]. Efficacy of anti-IgE (omalizumab) antibody treatment in allergic asthma was predicted by BAT [45]. Moreover, the more rapid clinical response to subcutaneous grass pollen immunotherapy, compared to oral immunotherapy, was reflected in BAT [45]. Importantly, side effects during allergen immunotherapy were predicted by sensitivity of basophils to insect venom [46].

In several allergic diseases, the outcome of BAT is related to severity. In allergic asthma, 'CD-sens' was related to both allergen-induced respiratory symptoms, as well as methacholine challenge test response [47]. Children with severe allergic asthma had higher basophil sensitivity to allergen in the BAT [48]. In allergic rhinitis, nasal symptoms were correlated to basophil sensitivity to allergen [49]. In peanut allergy, BAT showed high sensitivity and specificity (albeit with a low positive predictive value) to identify patients with severe reactions

to peanut during oral food challenges. These findings were consistent across different patient cohorts, which included some highly sensitised children [40, 50]. In a smaller peanut allergy study of children challenged as per routine clinical practice, thus with low-level sensitisation, BAT did not distinguish between mild and severe peanut allergy [51]. This lack of relation to severity was also demonstrated in the evaluation of lipid transfer proteins (LTP) allergic patients probably due to the pollen co-sensitization [52]. However, in a recent study of egg allergy, the BAT predicted severe reactions to baked egg, while no significant difference could be found in terms of demographic, clinical or other immunological parameters. These findings reinforce the premise that BAT most closely reflects allergic reactions to egg and thus could provide information on severity [18, 53].

2.2 | From Venoms to Food and Drug Testing

Hymenoptera venom allergy (HVA) is one of the most serious IgE-mediated HSRs due to the high risk of severe and even fatal anaphylaxis. In most patients, HVA can be effectively treated by venom-specific immunotherapy (VIT). However, correctly identifying the clinically relevant venom is still a challenge given the pronounced cross-reactivity between venoms [6, 33]. In HVA, BAT has proven an effective tool in identifying the primary sensitizing antigen, monitoring ongoing VIT, and successful tolerance induction [54–56], as well as predicting a higher risk of side effects [46, 57, 58]. However, BAT is still not currently part of the routine diagnostics in all patients [59] nor is it included in the EAACI guidelines on allergen immunotherapy for HVA yet [33].

Food allergy diagnosis can significantly impact patients' and their families' lives. Therefore, accuracy in diagnosis is essential for all suspected foods. The diagnostic work-up starts with the clinical history to allow for identification of the appropriate allergens for testing and the interpretation of the clinical relevance of the test results. Skin prick tests (SPT) and sIgE to extracts have high sensitivity, whereas sIgE to components and BAT also have high specificity to support the diagnosis [32]. Thus, current Food Allergy European Academy of Allergy and Clinical Immunology (EAACI) Guidelines [37] recommend that SPT and sIgE to allergen extracts be used as first-line tests, and sIgE to individual allergen molecules as second-line. If available, BAT can be undertaken in the equivocal cases before performing time-consuming and potentially risky oral provocation tests, should standard allergy tests be insufficient to provide a diagnosis [32]. The percentage of CD63+ of anti-FcεRI+/CD203c + identified basophils assessed on BAT has been reported as useful for distinguishing allergy versus sensitization to galactose-α-1,3-galactose (alpha-Gal/α-Gal) [60], shrimp, sesame, and peanut allergens [32, 61, 62]. Proposed clinical applications of the BAT in food allergy have been very recently reviewed in [24].

Immediate drug HSR (iDHSRs) diagnosis is currently based on clinical history and skin tests (STs), which in some cases cannot be done due to toxic effects of drugs on skin, in particular chemotherapy agents and, for some drugs, low sensitivity [63, 64]. It is noteworthy that iDHSRs do not necessarily require a sIgE-dependent response involving a drug-specific activation of the adaptive immune system. They can also result from alternative specific and nonspecific mast cell and basophil activation and degranulation, such as complement-derived anaphylatoxins and off-target occupancy of mast cell and/or basophil surface receptors such as the MRGPRX2 [3, 65] or even entirely independently of mast cell and basophil degranulation, as observed in hypersensitivity to nonsteroidal anti-inflammatory drugs [3, 65]. Therefore, in vitro/ex vivo flow-cytometry– based tests such as the BAT can complement STs to confirm a diagnosis of iDHSRs, allowing for the evaluation of a wide panel of drugs (importantly not only the active ingredients, but the excipients and/or metabolites, which are not possible to evaluate as independent components in humans). The BAT may therefore greatly improve both the sensitivity and specificity of diagnosis and aid in the identification of underlying mechanisms [66]. Additionally, BAT is useful to assess potential cross-reactivity between drugs with similar chemical structures, which is especially relevant when safe alternative equally effective therapies are sought [19, 67, 68].

There is increasing evidence that cell-based tests such as BAT appear to be able to determine the suitability and consequently safety of biologicals, therefore permitting the selection of a potential alternative biologic with sufficient certainty [69, 70].

3 | The Challenge of Allergic Reactions in Clinical Oncology

Treatment-emergent adverse events (TEAEs) and HSR up to anaphylaxis to cancer therapeutics must be constantly managed in clinical oncology. The risk for HSR is potentiated by multiple treatment cycles. These TEAEs may be either infusion-related, IgE or non-IgE mediated HSR, or immune-related adverse events (irAEs), the latter often linked to improved overall survival [71–73]. Chemotherapeutics, such as platinum derivatives and taxanes, are the most common triggers [74–76]. Hormonal therapies are associated with TEAEs, for instance in non-metastatic or metastatic prostate cancer where skin rashes occur in 20%–30% of cases [77]. The same study identified pre-existing allergy or atopy as specific risk factors. Positive ST reactions were proposed as biomarkers in antineoplastic hypersensitivity in an observational, retrospective study on mostly ovarian cancer patients [78]. Therefore, it may be beneficial for allergy and atopy testing to be included in oncology practice prior to treatment for risk stratification.

Overall, protocols to treat HSR to anti-cancer therapeutics range from symptomatic treatment to rapid drug desensitisation (DD, in which tolerance to the therapeutic is achieved by controlled administration of the drug dose [79]) and tolerance induction. Nevertheless, new tools such as BAT for predicting and monitoring TEAEs and responses to premedication or DD may add significant value.

4 | The BAT Translated From Allergy in Oncology

4.1 | Chemotherapy and Immunotherapy

BAT has proven useful in assessing allergic reactions to chemotherapy agents such as platins, taxanes, and some monoclonal antibodies [80–83]. Importantly, special handling of chemotherapies for ex vivo testing is recommended (Box 1).

BOX 1 | Challenges in Handling Chemotherapy Drugs for In Vitro Tests Including BAT.

- Drugs should be prepared and diluted by the Hospital's pharmacy in controlled and safe conditions.
- The chemotherapy drug should be kept in syringes within a sealed opaque envelope stored in the dark to prevent degradation and kept at 4°C until use, properly labelled including the name of the drug, concentration and date of preparation.
- In the laboratory, and during the acquisition of blood cells using flow cytometry equipment, besides general laboratory PPE (lab coat and nitrile gloves), additional equipment is required including sterile surgical gloves, a single-use waterproof coat, and an FFP2 mask.
- The complete processing of these samples, as well as drug dilutions, should be carried out in a laminar flow hood.
- Once the drug has been diluted and the BAT stimulation performed, tubes should be placed in suitable racks, duly capped both for incubation at 37°C and centrifugation. After centrifugation, it is recommended to wait before uncapping the tubes to allow the aerosols be settled.
- All materials in contact with the drug (including gloves and disposable PPE) must be discarded in a waste safety disposal container specifically for cytotoxic and cytostatic drugs and protected by a double hermetic seal.

Increased expression of CD203c in IgE-mediated reactions to carboplatin has been linked with overexpression of FcεRI [84]. In 15 patients and 6 healthy controls, BAT showed sensitivity of 73% and specificity of 100% in the diagnosis of reactions to specific platinum compounds in allergic patients [85]. Although increased expression of CD203c and CD63 was observed in these patients, higher expression of CD63 was measured in severe reactions. In a prospective study of 121 patients with suspected immediate chemotherapeutic HSR presenting different reaction phenotypes (i.e., type I, cytokine release syndrome (CRS), and mixed phenotype symptoms), BAT showed 79% and 50% sensitivity in type I-IgE-mediated reactions and mixed reactions to platinum salts, respectively, with a high specificity (100%). High correlation with ST was also observed and considered a promising tool for delabeling and endotyping platinum salts type I HSR [86]. In a study on 15 patients diagnosed with anaphylaxis to paclitaxel and docetaxel, BAT showed 53% sensitivity and 87% specificity for CD203c, while these rates were 33% and 87% for CD63, respectively [81]. Higher sensitivity was recorded in patients with positive ST, suggesting alignment with IgE-mediated endotypes.

The mammalian expression system used for the generation of monoclonal antibodies could also influence the propensity for triggering type I HSR. For example, monoclonal antibodies such as the anti-EGFR chimeric antibody cetuximab, generated in murine SP2/0 cells, are decorated with the carbohydrate α-Gal, found on proteins and lipids of non-primate mammals. Moreover, α-Gal can be present in foods, such as red meat and mammalian (product)-based medications. In patients previously sensitised to α-Gal, severe TEAEs can be caused upon first cetuximab infusion [87]. Consequently, testing for HSR to α-Gal, including cetuximab, with BAT prior to treatment may add value to risk evaluation [88, 89]. For several other anti-cancer biologicals (i.e., alemtuzumab, atezolizumab, bevacizumab, and necitumumab), HSR reactions have been described (including anaphylaxis) [90–92]. Therefore, the BAT could be a useful tool in selecting patients who could be prioritised to receive cetuximab treatments, while others may be offered alternative therapies. In future, it may be possible to develop drugs with lower propensity to trigger hypersensitivity reactions, thus the BAT could eventually lead to the optimisation and selection of appropriate biologic treatments. Given the risk of severe reactions to certain cancer therapeutics, identifying patients at risk and managing these reactions is crucial. One promising approach to address this is drug desensitisation.

4.2 | BAT in Drug Desensitisation for Oncology Patients

DD is a cornerstone procedure in the management of patients allergic to chemotherapies, biologics, antibiotics, and small molecules. DD is used to maintain first-line treatments, with reports of up to 95%–100% success rate for rapid DD achieved that enables safe and effective use of taxanes, platins, and monoclonal antibodies in patients with a previous reaction [75]. Thus, it has a positive impact on patient outcomes and quality of life [93].

As a high-risk immune therapy modality, it requires accurate diagnosis based on phenotypes and endotypes, risk stratification, and personalised protocols. Biomarkers are essential to identify endotypes, address the mechanisms of reactions, and importantly, monitor DD response by assessing the decrease of basophil sensitivity towards the drug [82, 85, 94, 95]. Beyond diagnosis, BAT may help to monitor the immunomodulation induced by DD in allergic patients to cancer therapeutics and the risk of suffering breakthrough reactions (BTR) during the procedure.

Three cases of allergy to brentuximab, a chimeric monoclonal anti-CD30 antibody for Hodgkin lymphoma and systemic anaplastic large cell lymphoma, confirmed by ST and positive BAT, showed negative test results after successful DD. However, premedication with corticosteroids was administered at the beginning of the procedure, which may impact the BAT result [95]. Interestingly, the positivity of an initial BAT was significantly associated with a higher risk of suffering BTR during DD. Twelve out of 13 patients who suffered a BTR presented an initial positive BAT with an increased expression of CD203c in 12 (92%) and CD63 in 9 (69%) of patients, as compared to those without BTR, in which BAT was positive. In one patient, BAT positivity increased progressively during DD, which correlated with the occurrence of BTR. In contrast, the expression of both CD203c and CD63 decreased dramatically during the last DD, coinciding with the absence of reactions. Similar observations have been reported for the anti-CD20 antibody rituximab [96] and the anti-HER2 antibody pertuzumab [82]. While ST remains the gold standard for the evaluation of chemotherapy drugs hypersensitivity reactions, many drugs cannot be used due to their vesicant effects on patients' skin. This limitation is not applicable to BAT, which does not have in vivo toxic impact.

Therefore, BAT seems to be a promising tool for monitoring the immunomodulation achieved by DD protocols and for identifying patients at risk of suffering BTR during this procedure [85, 97]. Its implementation may also enable the safe reintroduction of the culprit drug through desensitisation. Currently, BAT is proposed as a diagnostic tool in the algorithms for evaluating chemotherapy reactions [74]; however, its use has been limited to research and its implementation in clinical practice requires further standardisation and validation.

4.3 | Basophils and Cancer Outcomes

Basophil activation states can be influenced by type 2-biased and alternative type 2 inflammatory signals, which are also features of cancer. Yet, the understanding of the role of basophils in the development and prognosis of cancer has, until recently, received little attention. Basophils have been identified within the TME, including in lung adenocarcinoma [98], pancreatic ductal adenocarcinoma [99], cholangiocarcinoma, thymoma, and renal tumours [100]. Recent studies revealed differential associations between basophils and patient survival outcomes. Higher expression of gene signatures for basophils and activated basophils in ovarian, sarcoma, endometrial, lung, and breast tumours was associated with improved patient outcomes, whereas activated basophils in gastric tumours were associated with a worse prognosis [13, 100]. Higher blood basophil counts associated with improved survival outcomes in glioblastoma [101] and colorectal cancer [102], and BAT has been utilised to show that higher proportions of basophils, and circulating basophils with a greater capacity for activation ex vivo, were associated with improved overall survival of ovarian cancer patients [13]. The incidence of "non-releasing" basophils to ex vivo IgE and non-IgE mediated immune stimulation in this ovarian cancer patient cohort was comparable to that described in other groups of patients whose basophils were subjected to IgE-mediated stimulation using the BAT. Such examples include children evaluated for peanut allergy, individuals with tree and grass pollen allergies, and cow's milk intolerance [13].

Overall, the presence and activation of basophils in tumours or the circulation of cancer patients may be associated with improved or poorer survival outcomes, depending on the influence of the local inflammatory milieu in the TME of different cancers.

4.4 | BAT in the Development of Novel Therapeutics

In the field of AllergoOncology, BAT has more recently been implemented as a tool to study emerging anti-cancer treatments, such as tumour-targeting IgE therapeutics. IgE isotype antibodies could provide significant advances to the treatment of cancer that may complement IgG antibodies almost exclusively used for treatment presently [103]. However, due to known functions of IgE antibodies in allergy and type I HSR, there is a perceived risk of anaphylaxis with these novel immunotherapies. IgE immune complexes could be formed with the presence of tumour-associated antigens and/or autoantibodies on mast cells and basophils, which could trigger degranulation and induce systemic reactions. As BAT is performed with unfractionated whole blood, any such potential mediators of basophil activation that may be found in circulation are present alongside test therapeutics ex vivo, making this an attractive model in which to study the propensity for triggering type I HSR by anti-cancer IgEs.

Having confirmed the capacity for basophil activation following ex vivo immune stimulation in blood from melanoma and ovarian cancer patients, it has been shown that exogenous IgE binds unoccupied FcεRI on the surface of these cells and forms immune complexes with multimeric antigens to trigger basophil activation [13, 104, 105]. BAT was therefore utilised to study the potential of anti-cancer IgE antibodies to mediate type I HSR. To date, four anti-tumour IgE antibodies have been evaluated, with no basophil activation observed following ex vivo stimulation with anti-CSPG4, SF-25, and trastuzumab -IgEs, and $<2\%$ of ovarian cancer patient blood samples being reactive to anti-MOv18 IgE ex vivo [104–108].

In addition to these pre-clinical studies, BAT was implemented as a HSR monitoring companion, alongside other clinical safety parameters, in the first-in-class, first-in-human phase I clinical trial of MOv18 IgE. Here, a baseline BAT before treatment predicted the single anaphylactic reaction to MOv18 IgE intravenous infusion, which was not predicted by SPT. Subsequently, sensitivity to MOv18 IgE in a baseline BAT was used as an exclusion criterion to ensure that no further patient with reactive basophils received treatment [106].

Therefore, the evaluation and clinical translation of anti-cancer IgE antibodies constitute another emerging application for BAT.

5 | Unmet Needs, Recommendations and Future Vision for BAT

5.1 | Unmet Needs and Recommendations for BAT

Over the past decade, BAT has gained recognition as a valuable in vitro diagnostic tool for HPS reactions to various allergens, including food, hymenoptera venoms, and drugs. Its utility has also extended to monitoring allergen immunotherapy, assessing natural disease resolution, and evaluating clinical responses to immunomodulatory treatment. Nevertheless, the application of BAT in oncology is limited in comparison to the field of allergy (Figure 2). The BAT has been recently explored in oncology for assessing hypersensitivity reactions to therapeutic agents such as platinum compounds, taxanes, and monoclonal antibodies, including the safety of anti-cancer IgE antibody therapeutics (Box 2, Figure 2). Building on insights gained from applications in allergy, additional evidence is required to establish the value of BAT in oncology, and several challenges in implementation, harmonisation, and wider applicability remain (Box 2, Figures 3 and 4).

In the emergency setting, TEAEs and HSR, including anaphylaxis to cancer therapeutics such as chemotherapy agents and monoclonal antibodies, present significant challenges. Identifying the underlying mechanism and optimising patient management remain complex, prompting the development of clinical oncology

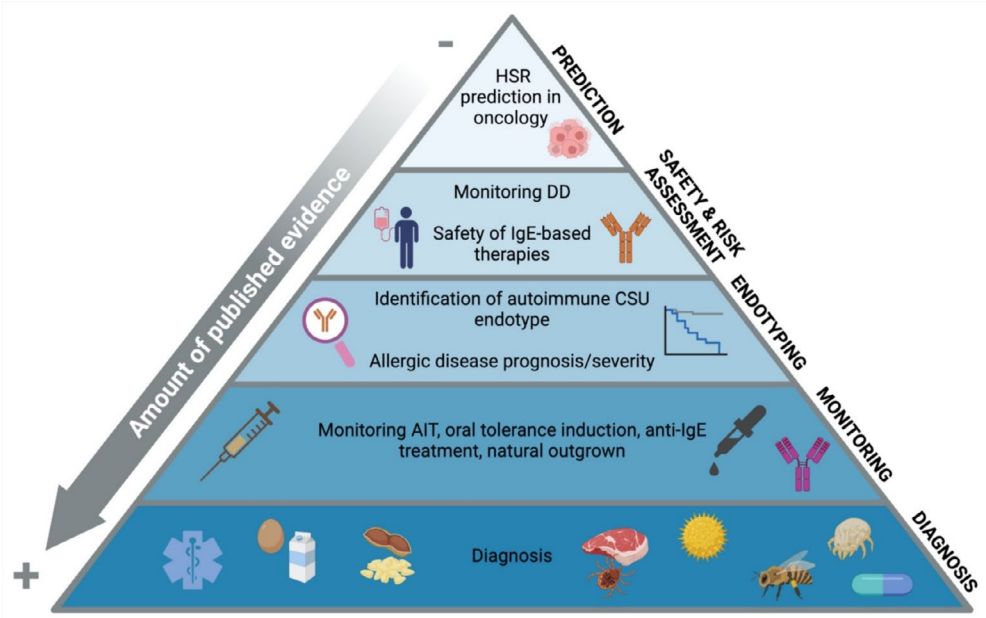

FIGURE 2 | **Hierarchy of published evidence for the use of the BAT**. This illustrates the hierarchy of evidence supporting the basophil activation test (BAT), from the most substantial evidence at the base to the least at the peak. **Diagnosis**: BAT is most strongly supported in the diagnosis of allergies, particularly food allergies (e.g., HSR to milk, egg, peanut, mammalian meat, and sesame), as well as drug and venom allergies. **Monitoring**: Evidence supports the use of BAT in monitoring allergen immunotherapy (AIT), oral tolerance induction, anti-IgE treatments, and the natural progression or resolution of allergy. **Endotyping**: Fewer studies apply the BAT to identify disease endotypes (e.g., autoimmune CSU) or assess allergic disease prognosis and severity. **Safety & Risk Assessment:** Emerging evidence supports using BAT to evaluate the safety and risk of therapeutic agents, such as during drug desensitisation (DD) protocols, or with novel IgE-based therapies or other anti-cancer agents. **Prediction:** BAT shows potential for predicting HSR, particularly in oncology settings, with the least published evidence among these applications. Created with BioRender.com.

guidelines [72] and best practice recommendations [109] to facilitate more effective and timely clinical interventions. While prophylactic premedication with dexamethasone and diphenhydramine is widely used to prevent such reactions, it remains to be determined whether BAT could help identify patients at heightened risk of reactions to different types of anti-cancer therapies and guide personalised treatment strategies.

Indeed, BAT has demonstrated utility in predicting safe therapeutic alternatives following HRS. However, protocols are labour-intensive, and the mechanisms of DD and its immunological effects remain poorly understood. While BAT has been used to monitor DD by assessing decreased basophil sensitivity to the drug [94] and to predict reactions during DD protocols [82, 85, 95], data are limited and sometimes controversial. Standardising BAT procedures and clearly defining testing conditions are essential for improving reliability and expanding clinical applicability (Figure 4). Notably, initial BAT positivity has been associated with an elevated risk of BTR during DD [82, 86], suggesting that further exploration could establish BAT as a valuable in vitro tool for stratifying DD risk and ensuring safety during these interventions. Whether BAT can effectively monitor the DD process over time remains an open question. Thus, a deeper understanding of the immunological effects of DD and the comprehensive performance characteristics of BAT are needed (Box 3).

The implementation of BAT is expanding in research and routine diagnostics. Despite its emerging use in oncology drug reactions, standardisation of BAT has proven difficult due to variability in basophil responses to the different drug classes [110]. Harmonisation and standardisation of protocols and test outcome analysis, with the scope to apply data-driven programmatic approaches [111], are essential for adaptation to oncology settings (Box 3). Further phenotyping of basophils, including their resting state (e.g., using CD203c) as well as deeper assessment of CD203c and CD63 expression under different stimulation conditions, must be assessed in relation to applications in oncology.

To further optimise the BAT for application in oncology, key aspects requiring clarification include determining the minimum number of basophils necessary for a valid test and minimum stimulation levels that would reflect a clinical reaction. These likely depend on the stimulant evaluated and the magnitude of activation that can be achieved ex vivo [112]. Additionally, the volume of blood required per test may need adjustment, particularly in patients with low basophil counts such as when following a suspected HPS reaction, and in neutropenic patients with advanced malignant disease. Another challenge relates to the requirement for fresh blood sample processing, normally within 24 h of blood collection. 'Non-releasers' in BAT pose an additional challenge; although the proportion of 'non-releasers' in oncology cohorts appears comparable to other patient cohorts [13], it remains unclear whether the basophil activation profiles of oncology patients differ significantly from allergic individuals, and thus whether these might potentially affect test outcomes.

BOX 2 | Perspectives and Key Considerations in Current Applications of BAT in Oncology.

Current applications of BAT in oncology

- Chemotherapy allergy:

Supports diagnosis of allergic reactions to chemotherapy agents (e.g., platins and taxanes) alongside SPT and sIgE tests.

- HSR to α-Gal and cetuximab:

Confirms α-Gal-related HSR (e.g., cetuximab) using BAT, complementing anti-α-Gal ELISA; and may be implemented before initiation of treatment to evaluate risk of reaction.

- Immunomodulation monitoring:

Tracks immunomodulation during DD protocols and identifies patients at risk of BTR during DD procedures.

- Disease endotyping and prognosis:

Identification of disease endotypes (e.g., autoimmune CSU) or assessment of allergic disease prognosis and severity.

- Predictive tool for IgE-based therapies:

Predicting HSR reactions to anti-cancer IgE antibody therapeutics.

Knowledge acquired from current implementation of BAT in oncology

- Basophils from cancer patient basophils can be identified and activated ex vivo by IgE and non-IgE mediated stimuli.
- BAT monitors type I HSR to a wide range of oncology drugs.
- Blood samples should be processed within 24 h.
- The proportion of 'non-releasers' among oncologic patients is comparable to other allergy cohorts.
- BAT can be adapted for wide and routine use in oncology settings.

Practical considerations for establishing BAT in oncology

Challenges related to samples:

- Difficulty obtaining blood from treated oncology patients for research, validation, and standardisation.
- Numbers of basophils sufficient for BAT that can be achieved from the blood samples of often neutropenic cancer patients.
- Requirement for fresh blood processing.

Technical and analytical needs:

- Deeper assessment of activation markers expression and/or upregulation under different stimulation conditions.
- Standardisation of the cut-off level for defining basophil activation and the magnitude of activation triggered (likely dependent on the stimulant).
- Standardisation of drug concentration for testing, this would need optimization and should beadapted to the nature of the chemotherapy agents.

Treatment impact:

- Prior cytolytic effects of chemotherapeutic therapy or immunomodulatory treatments, like high-dose systemic corticosteroids, on the function and fitness of basophils from cancer patients.

5.2 | Future Vision for the Application of the BAT

With regard to chemotherapeutics and biologics, BAT offers an opportunity to enhance the assessment of IgE mediated-allergic reactions across a broad spectrum of chemotherapy agents and monitor interventions aimed at achieving drug tolerance. In the future, BAT could be broadly applied in a clinical setting to predict type I HRS, and perhaps help distinguish HRS from other reactions such as cytokine release syndrome where BAT show negative results, or discriminate between IgE or non-IgE dependent activation of basophils by including specific stimulator arms to the conventional BAT, like anti-IgE biological agents (omalizumab), IgE-FcεRI disruptors (DARPins), or protein Bruton's tyrosine kinase inhibitor (ibrutinib), that could indirectly confirm the IgE-mediated activation of basophils [113–115]. Thus, BAT offers the opportunity to unreveal mechanistic insights unreachable with in vivo testing (i.e., skin testing and/or oral challenges) [19]. To this end, incorporating allergy and atopy testing as translational research tools for application into the oncology workup prior to treatment initiation is essential for effective risk stratification and clinical management.

HRS can occur even upon first exposure to cetuximab. BAT can predict the risk of cetuximab-induced anaphylaxis and reaction severity [89]. Moreover, α-Gal-sensitised individuals exposed to medications, vaccines, or medical instruments containing α-gal via oral, injection, transplantation, or surgical routes are at risk

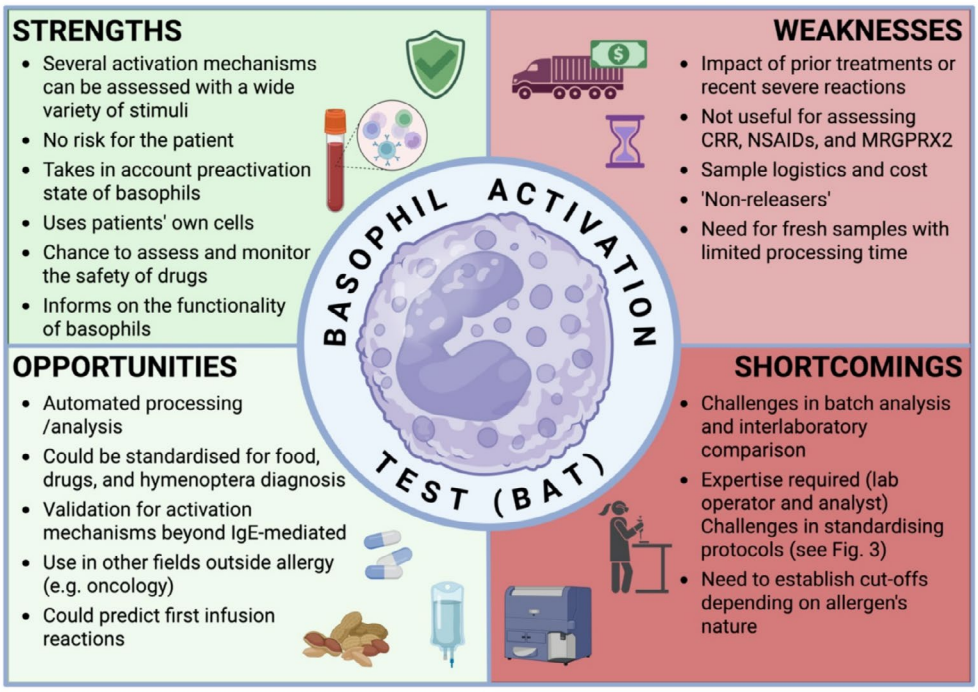

FIGURE 3 | **SWOT analysis of the Basophil Activation Test (BAT) as a clinical assay.** Using the SWOT analysis, we evaluate the feasibility of establishing the BAT as a routine clinical assay by analysing its Strengths, Weaknesses, Opportunities, and Threats (termed Shortcomings here) (SWOT). Strengths: Highlights the advantages of BAT over other in vitro/ex vivo assays and its successful clinical applications. Weaknesses: Identifies intrinsic challenges of BAT that limit wide implementation in the clinic. Opportunities: Explores areas for expanding BAT applications. Shortcomings: Examines current limitations of the BAT to be overcome. Created with BioRender.com.

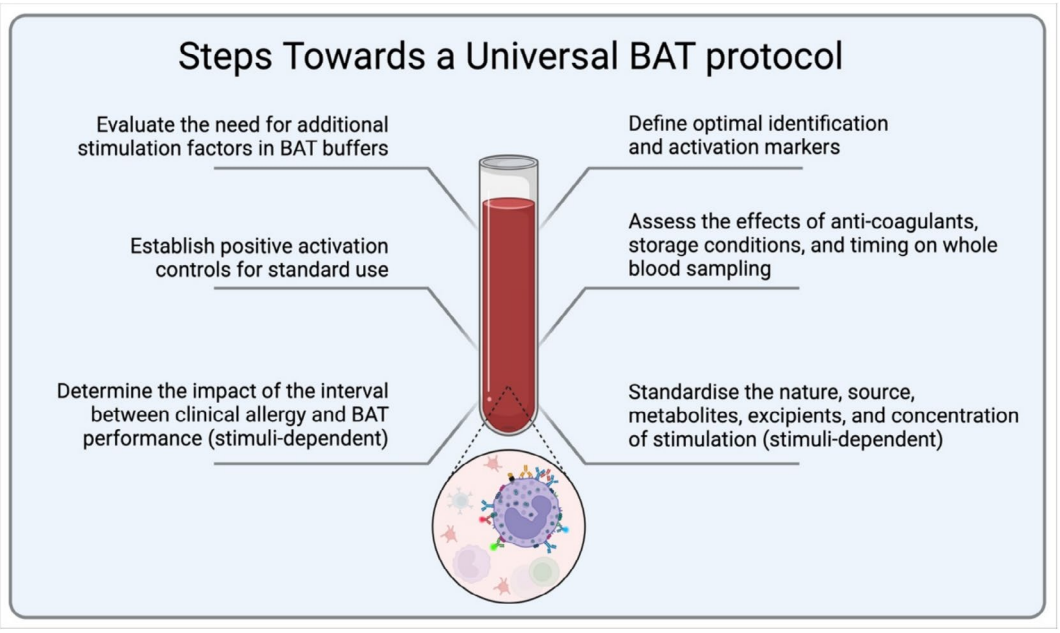

FIGURE 4 | **Challenges and suggested steps towards a universal and standardised BAT protocol**. We highlight key challenges and actions to achieving standardisation in the basophil activation test (BAT), including: Buffers and Stimulation Factors: Lack of information in commercial kits regarding buffer composition and insufficient studies on their impact on basophil functionality in BAT assays; Markers and Positive Controls: Variability in the identification and activation markers used, as well as inconsistencies in the positive controls provided by kits, including the absence of details on antibody composition and clone specificity; Blood Sample Collection: Lack of consensus on optimal anti-coagulants (e.g., EDTA vs. heparin), their concentrations, and standardisation of handling protocols, including the timeframe from blood collection to assay execution and the use of fixation for delayed analysis; Timing Post-Allergy Event: Uncertainty about the optimal interval between a clinical drug allergy reaction and BAT application, further complicated by drug-specific pathways that may affect test applicability; and Stimuli Concentration and Composition: Variability in the standardisation of basophil activation stimuli, including allergen or drug concentration, source, metabolites, and excipients, posing challenges to protocol harmonisation. These factors underscore the focus areas for further research and consensus-building to develop a robust, standardised BAT protocol. Created with BioRender.com.

of developing severe reactions [116]. The α-Gal cetuximab association mandates the need to better understand broader HRS arising from cross-reactivity between diverse antigenic or allergenic sources, and this will also further expand the diagnostic and mechanistic relevance of BAT.

BAT may aid drug development, particularly the safety of biologics, including managing the safe administration of anti-cancer IgE antibodies in clinical trials. Future applications may include investigating the mechanisms underlying an individual's sensitivity to a particular IgE or the broader IgE immunotherapy class. For now, the use of unfractionated whole blood in BAT enables the simultaneous assessment of circulating antibodies of various isotypes. This could be applied to study antibody-mediated and cell-intrinsic responses. This positions BAT as a valuable platform for studying anti-cancer IgE therapeutics and other innovative immunotherapy approaches (Figure 3).

BAT offers a distinct advantage over other flow cytometry-based cellular tests like the Mast Cell Activation Test (MAT) by leveraging patient autologous whole blood, which preserves the full spectrum of circulating immune cells and potential cross-linking factors within the context of the patient's disease. This approach is particularly valuable in oncology, where the circulating milieu is complex, different from that in healthy or allergic states, variable depending on the malignant disease context, and thus difficult to replicate using in vitro models such as LAD-2 cell lines or mast cells derived from human haematopoietic stem cells. Emerging developments may instead include an "extended BAT" capable of assessing the activation of additional granulocytes, such as neutrophils and eosinophils, which also upregulate CD63 upon activation [9]. T cell testing has been classically employed for T cell-mediated non-immediate HSR; however, given the involvement of Th2 cells in the class-switching to drug-reactive IgE antibodies, it has also been recently explored in IDHRs. Flow cytometry-based T cell activation tests (TATs) have recently emerged as promising in vitro test methods with the potential to address some current diagnostic limitations in IDHRs [66].

Beyond oncology, BAT shows promise for analyzing basophil activation and correlation with clinical outcomes, though evidence

BOX 3 | Vision for Implementing BAT in Different Oncology Applications in AllergoOncology.

- **Current opportunities for implementing BAT in oncology**.
- Allergic reactions and drug tolerance: BAT presents opportunities to:
- (A) improve assessment of allergic reactions to a broader range of chemotherapy agents
- (B) monitor interventions to achieve drug tolerance.
- Compounds not available for in vivo use: BAT offers the opportunity to test compounds not available for in vivo use, such as certain excipients. Facilitates discerment of reactions initiated by the active compound from those triggered by excipients.
- Drug development: BAT offers additional value in drug development to study the safety and functional effects of biologics, inform patient stratification, and optimize biological agents less likely to trigger hypersensitivity reactions.
- Cross-reactivity and HPS: BAT can be useful to understand further HSR raised from cross-reactivity phenomena between different antigenic/allergenic sources.
- First infusion HSR prediction: BAT has a promising value in the prediction of the first infusion HPS reaction and assessing its mechanism of action. Future applications should include prediction of type I HPS in the clinic for improved risk management and patient safety.
- **Current needs for implementing BAT in oncology**
- Global harmonisation and standardisation: Harmonisation across laboratories worldwide and standardisation of the BAT for any given application are needed for concrete protocols to be adapted to the oncology setting.
- Basophil activation in oncology versus allergy: Whether the basophil activation profile in oncologic patients is the same as in allergic patients, and whether this may have implications on the test outcome remains unknown.
- Understanding HPS activation pathways: BAT is an evolving test to be adapted to cover the current gaps in understanding activation pathways that lead to HSR.
- Monitoring DD process: Whether BAT can contribute or not to monitoring DD process over time requires further understanding of the immunological impact of this procedure and a more complete understanding of the performance of the test.
- DD risk stratification and safety: Further exploration will position BAT as a useful in vitro tool for DD risk stratification and safety of the DD procedure. There is a need to better define and standardise the conditions under which the test can be conducted.
- Basophil activation in health and disease: Basophil function is widely studied in allergy; however, there is still a lack of full understanding of basophil activation in health and other contexts of disease (cancer, immune dysregulation i.e. CSU, immunodeficiency, and autoimmunity), and BAT can be useful for this purpose.
- Basophil subpopulations and activation propensity: Basophil subpopulations have been identified, but it is not clear how this may relate to basophil activation propensity across diseases and how this might impact the interpretation of data from the BAT.
- Need for larger studies: Larger and systematic studies providing real-world evidence are required to strengthen future use of BAT in the perspective of AllergoOncology.

remains limited [13]. While basophil function has been widely studied in allergy, its activation dynamics in other disease contexts, such as cancer, immune dysregulation, immunodeficiency, and autoimmunity, remain poorly understood.

Addressing these limitations and key unknowns will allow BAT to be widely applied in AllergoOncology for research, monitoring, and diagnosis of type I HRS. This will aid the stratification of patients, allow for administration of potentially lifesaving treatments, and inform the development of novel approaches. This utility underscores its potential as a pivotal tool in personalised medicine and exemplifies the importance of interdisciplinary research and cooperation to join expertise in allergy and oncology for the benefit of patients.

An in-depth study of emerging applications of the BAT in oncology is required. Several applications of the BAT in oncology have been informed by and adapted in the field of AllergoOncology from allergy to oncology, in translational cancer research, studying the mechanisms of HPS reactions to cancer therapies, and to aid diagnosis, therapy monitoring, and risk stratification. These evaluations are and will continue to be of utmost importance for disease management and offer major opportunities for collaboration between allergy and oncology experts. We advocate the need for enhanced focus on addressing standardisation challenges and leveraging these outputs for precision medicine. By linking allergy and oncology, the key remaining limitations can be addressed, with the aim of realising the significant promise of the BAT as a robust tool to enhance personalised care in Allergy and AllergoOncology.

6 | Conclusion

Here, in this position paper, we explore and advocate the potential of the BAT as a valuable tool in the field of AllergoOncology. We review its emerging applications, which have been informed by and adapted from allergy to oncology. These applications span basic, translational, clinical research, patient stratification, and therapy development. We discuss the current challenges of implementing BAT in oncology, and we highlight the importance of and opportunities for collaboration between allergy and oncology experts. Capitalising on long-acquired expertise on the development of BAT for applications in allergy, we propose key research directions and routes to achieve translation and clinical application of this highly promising allergy tool as a validated and widely used assay for AllergoOncology research, therapeutics development, clinical management, and ultimately patient benefit. We advocate the need for enhanced focus on addressing standardisation challenges and leveraging outputs for precision medicine. By linking allergy and oncology, the key remaining limitations can be addressed, with the aim of realising the significant promise of the BAT as a robust tool to enhance personalised care in allergy and AllergoOncology.

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Conflicts of Interest

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Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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