



Precision medicine in cow's milk allergy

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Purpose of review

The aim of this review is to describe the role of precision medicine in the diagnosis, treatment, and monitoring of cow's milk allergy.

Recent findings

The development of 'omics' sciences in the field of food allergy has led to a better understanding of the allergenicity of cow's milk proteins and significant advances in the knowledge of the pathogenesis and mechanisms of cow's milk allergy. Omics-based technologies allow the practitioner to better differentiate cow's milk allergy subtypes and to predict cow's milk allergy (CMA) persistence over time. Precision medicine extends the role of the oral food challenge, to determine the individual's threshold doses, and to establish tolerance to baked milk products. Other than symptom relief, dietary strategies are currently being investigated for the potential to induce tolerance. Oral immunotherapy offers a treatment option for patients with severe and persistent IgE-mediated CMA. Individual baseline-immune profiles may be predictive of cow's milk oral immunotherapy safety and efficacy.

Patient data derived from current technology, in combination with the patient's history, can be translated into treatments targeted at patient-tailored interventions.

Summary

The identification of novel biomarkers may improve diagnostic accuracy and also predict patient responsiveness to treatments. Integration of patient data will become increasingly important as omics technologies become more widely used in the clinical setting.

Keywords

biomarkers, cow's milk allergy, omics, patient data, precision medicine, tolerance

INTRODUCTION

Precision medicine is an emerging approach to studying disease pathogenesis, progression, and treatment, to obtain information specific to an individual that can be used to identify the optimal treatment choice for that patient [1] (<https://erj.ers-journals.com/content/50/4/1700391>).

The term 'precision medicine' has replaced the term 'personalized medicine,' which is still used interchangeably by most [2].

The personal approach that is an inherent part of the doctor–patient relationship is a central aspect of precision medicine.

However, the term precision medicine implies a novel concept of medicine that takes into account a wide array of individual data, including clinical, genetic, lifestyle, and biomarker information for each person to make the optimal treatment decision for that patient [3].

The development of omics sciences (Fig. 1) has led to a significant improvement of precision medicine in food allergy [4,5].

However, an accurate clinical history is and remains the primary diagnostic tool for all types of food allergy [6].

Cow's milk allergy (CMA) is an immunological mediated adverse reaction to one or more cow's milk proteins, which are normally harmless to a nonallergic individual [7]. CMA is one of the most common food allergies in infancy and the impact on individual well-being should not be ignored [8,9].

The clinical presentations of CMA vary in terms of type and severity, making it the most complex presentation of food allergy in childhood and challenging to diagnose. Symptoms of CMA, in particular

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Curr Opin Allergy Clin Immunol 2020, 20:233–241

DOI:10.1097/ACI.0000000000000640

KEY POINTS

Precision medicine is a novel approach aiming to deliver personalized care to patients. Applying precision medicine in diagnosis, treatment, and monitoring of cow's milk allergy, includes:

- The ability to make a precise and reliable CMA diagnosis, taking into account patient characteristics and individual immune/allergen profiles. Omics-based technologies allow the practitioner to better differentiate cow's milk allergy subtypes.
- Tailored-treatment including formula choice, and the understanding of individual threshold dose for small amounts of milk or baked milk.
- Novel treatment strategies, targeting gut modulation, are expected to promote tolerance induction. The administration of hypoallergenic molecules in a near future may increase the safety level of oral immunotherapy.
- The biggest target is to discover novel biomarkers that will improve diagnostic accuracy and to predict patient responsiveness to the treatments, enabling the clinician to choose the most appropriate treatment option for each patient.

non-IgE-mediated CMA, overlaps with lactose intolerance and common skin and gut symptoms in infancy, unrelated to CMA. Therefore, to diagnose CMA, the clinician needs to differentiate the symptoms relating to CMA from those which are unrelated. Making a correct diagnosis is crucial in order to avoid unnecessary milk avoidance, while also recognizing true allergy, especially potentially if life-threatening.

This review describes how practising precision medicine can assist the clinicians in making an accurate, timely, and precise CMA diagnosis, in predicting CMA resolution and in making tailored treatment decisions.

New insights into the allergenicity of milk proteins

Cow's milk contains caseins, accounting for approximately 80% and whey proteins accounting for approximately 20% of the total protein content. Caseins (Bos d8), the major cows' milk allergy allergens, include four isoforms in different percentages: α -S1-casein (Bos d9, 32%), α -S2-casein (Bos d10, 10%), β -casein (Bos d11, 28%), and κ -casein. Whey proteins consist of α -lactalbumin (Bos d4), β -lactoglobulin (Bos d5), immunoglobulins (Bos d7), bovine serum albumin (BSA, Bos d6), and traces of lactoferrin [10]. Caseins are very stable to heat treatment, as demonstrated by their presence after

60 min at 95 °C [11]. In contrast, whey proteins are thermolabile, with changes in their allergenicity, probably because of the destroying of conformational epitopes by heating [12].

The majority of cow's milk allergic children (70–75%) can tolerate extensively heated (baked) products [13].

Proteomics techniques yield new insights into the allergenicity of milk protein in baked milk products.

In a very recent study, the effects of heating and the presence of a wheat matrix on cow's milk allergenicity has been evaluated [14^{*}]. The analysis of the protein profile using proteomics showed that the final immunoreactivity of baked milk within a wheat matrix (e.g., muffin) appears consistently reduced compared with the heated milk (180 °C for 10 min), not included in a matrix. The only residual immunoreactivity of baked products was because of the resistant epitopes belonging to S1, as demonstrated by proteomic analysis. The results of this study confirm that the interactions between milk proteins and some components of the food matrix during heating play an important role in the reduction of allergenicity.

The effects of matrix other than wheat on milk proteins allergenicity remain to be investigated.

Precision medicine in the diagnostic work-up of cow's milk allergy

The clinical presentation of a food-induced allergic reaction depends on a number of variables (see Fig. 1).

Diagnosing CMA requires differentiating allergic symptoms from those not being triggered by a food allergy and identifying CMP in the case of allergen.

The diagnosis of CMA is relatively easy in case of patients who present with a history of immediate, acute and objective symptoms that can affect the digestive tract (diarrhea, vomiting), the skin (acute urticaria, angioedema), the respiratory tract (stridor, wheezing) or induce a systemic reaction (anaphylaxis) within 2 h after ingesting dairy products.

In cases of a high pretest probability, for example, clinical history of immediate symptoms and concomitant IgE sensitization (positive prick test and/or positive specific IgE (sIgE)), oral food challenge (OFC) may not be required [15].

In cases of moderate probability, which is true for the majority of cases, OFC is necessary to confirm a CMA diagnosis.

In clinical practice, component-resolved diagnostics may be useful for the evaluation of reactivity to baked milk, helping to identify different clinical

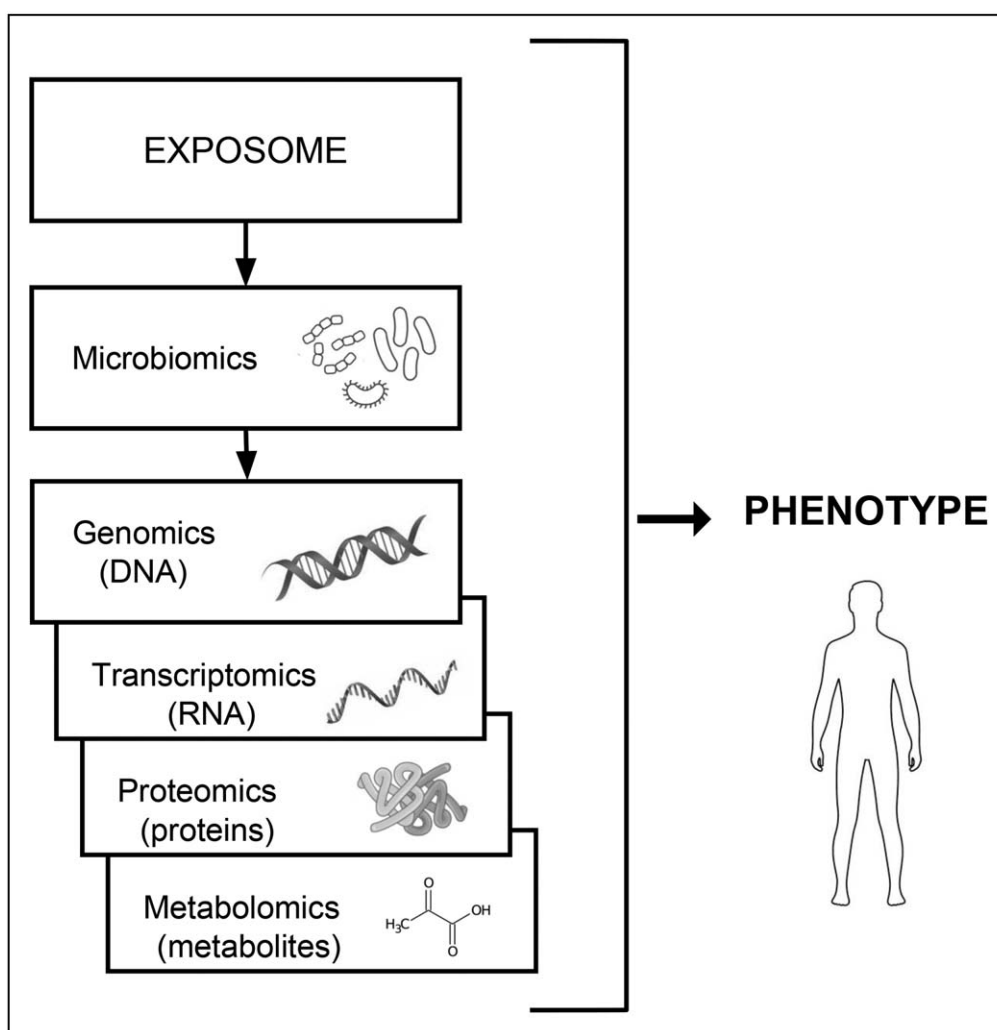


FIGURE 1. The omics sciences.

phenotypes on the basis of molecular patient-specific IgE profile [16,17].

Patients who exhibit higher levels of casein-specific IgE are more likely to react to baked milk [18]. Lesser basophil activations was also observed in patients who tolerated baked milk than those who reacted to heated milk [19].

Specific IgE-diagnostic decision points for CMA diagnosis (regular milk and heated milk, respectively) have been proposed [18,20].

The use of decision points, combined with the patient clinical history, may allow the physician to identify optimal candidates to undergo OFC with baked milk but often does not account for individual nuisance of the patient's history. These cut-off points may also be population specific and not relevant to all. It is important to note that none of the suggested decision points are 100% specific or sensitive.

Several studies have now highlighted not only the gastrointestinal symptoms commonly seen in children with non-IgE-mediated food allergy but

also the high prevalence of feeding difficulties, such as vomiting, constipation, and abdominal discomfort that should be considered in investigating food allergy.

Allergic dysmotility includes different conditions, for example, gastroesophageal reflux disease (GERD), dyspepsia, and constipation, where digestive motility is altered by the neuroimmune-muscle inflammatory interaction triggered by the cow's milk proteins in predisposed individuals [21,22]. It is estimated that up to half of the cases of GERD in infants younger than one year may be related to CMA on the basis of clinical presentation and improvement on cow's milk avoidance [23].

In case of gastroesophageal reflux, a combination of skin and GI symptoms increases the likelihood of CMA, although combination by coincidence is possible given the high incidence of both conditions in infancy.

OFC is considered the gold standard to diagnose CMA [24]. However, in young children, OFC is often

declined by parents of young infants or delayed because of the fear of a severe reaction or for other reasons. Furthermore, the clinical reaction during or after an OFC is sometimes difficult to interpret in infants and it is possibly related to mechanisms other than immune reactions [25[¶]]. Therefore, in young children with GI symptoms and negative allergy tests, a cow's milk-free diet is often started based on a subjective interpretation of clinical history and clinicians' experience. If the infant improves on cow's milk avoidance, it is highly important to try and convince parents to reintroduce CMP after a 2–4-week trial to confirm a diagnosis of CMA.

Hydrolyzed formulas may improve clinical symptoms by accelerating gastric emptying time [26], reducing mast cell mucosal infiltration, thus normalizing immune-nerve interactions and improving motor abnormalities [22,27].

As a result, in infants with gastrointestinal symptoms, CMA may be overdiagnosed because of other beneficial effects of hydrolyzed formulas, as opposed to the effect of CMP avoidance [28].

The improvement of symptoms with targeted dietary treatment could reduce the stress level of caregivers and healthcare costs and improve the quality of life of food-allergic infants and family [29].

Distinguishing reflux-induced esophagitis from eosinophilic esophagitis in children is also challenging [30].

In younger children, eosinophilic esophagitis may be suspected in case of association of proton pump inhibitor refractory gastroesophageal reflux (GER) to feeding difficulties and failure to thrive [31].

In patients with newly diagnosed eosinophilic esophagitis, differential expression of genes in association with esophageal fibrostenosis was found [32]. These findings may orient the development of targeted treatments and may distinguish which patients are more likely to respond to such therapies.

The oral food challenge in the era of precision medicine

OFC is considered the gold standard for the diagnosis of food allergy and to evaluate the acquired tolerance to CMP [33]. In the era of precision medicine, the purpose of OFC is changing.

It is expected of the clinician not simply to confirm or to exclude the diagnosis of CMA, but also to determine which is the patient threshold dose, to establish if the child is able to tolerate baked milk products or small amounts of milk. OFC is also necessary to identify the threshold dose for oral

immunotherapy; this indication is associated with more severe reactions during OFC [34^{¶¶}].

Molecular diagnosis and other functional tests may be useful to predict the severity of reaction during OFC. In this respect, a significant association has been found between anaphylaxis during the OFC with cow's milk and the sIgE levels for caseins [35], as well as with higher basophil reactivity [19].

More recently, some STAT (signal transducer and activation of transcription 6) gene variants have been associated with more severe allergic reactions during both peanut and cow's milk double-blind, placebo-controlled food challenge both [36]. The role of STAT6 genetic polymorphisms seems independent of the causal allergenic food; however, these results need to be further confirmed. To date, although some functional tests (e.g., Basophil Activation Test) seem promising [37[¶]], and combination of tools are under investigation [38], there is no reliable in-vitro test able to predict the in-vivo reaction.

Precision medicine in predicting cow's milk allergy persistence

Looking for prognostic markers, casein has been identified as the best option to discriminate between transient and persistent CMA [39], where higher levels of sIgE to casein correlates with CMA persistence [40,41]. Recently, the effects of individual genetic variants on the natural history of CMA have also been described. As an example, the A genotype in rs324015 has been demonstrated to be associated with an earlier age of developing tolerance for CMA [42]. Research in epigenomics has also identified novel and heritable changes in DNA methylation, associated with the development of natural tolerance in cow's milk allergy [43]. Dietary intervention could therefore modulate epigenetic changes affecting the immune system in CMA children, inducing tolerance development [44^{¶¶}].

Beyond omics, clinical data may be useful to predict persistence of CMA. Long-lasting CMA is characterized by the presence of the familial atopic disease, a longer period between the consumption of the CMP and the onset of symptoms, a high frequency of multiple food allergies, and reactivity to lower doses of milk [45,46].

Precision medicine in cow's milk allergy management

Allergen management of cow's milk allergy includes discussions on formula choice and other milk alternatives, tolerance of baked milk products and understanding food labels and threshold levels.

Table 1. Factors to take into account when choosing an alternative formula or commercially available milk

Infant formula (under 12 months)	
Age	Use infant formula under 12 months Use follow-on formula over 1 year Can use enriched/fortified commercial milks/beverages over 2 years (perhaps 1 year pending dietetic review and sufficient growth)
Growth	Use amino acid formula as if poor growth If enriched/fortified commercial milks/beverages are used (over 2 years); ensure that food intake is sufficient
Cost/insurance coverage	If formula is covered by insurance/healthcare system, continue to use as nutritionally required
Availability	If fortified enriched/fortified commercial milks/beverages are not available, use infant/follow-on formula If above is not an option, do ensure that food intake is sufficient – and nutrient supplementation is sufficient if needed
Taste	If child does not take, either formula or enriched/fortified commercial milks/beverages, look for other options such as changing the formula or use a different commercial milk/beverage
Parent preference	Parents may prefer to use enriched/fortified commercial milks/beverages rather than formulas. Parents may prefer, vegan/plant-based foods, etc.
Fortification	If dietary intake is not sufficient give preference to formula or fortified beverages. It is very important to realize that enriched/fortified commercial milk/beverages are fortified with vitamins and/or minerals BUT protein and calorie content is still inadequate to support growth in the absence of sufficient dietary intake of protein and calories
Food allergen	Avoid allergens in enriched/fortified commercial milks/beverages if allergic: soy, nuts, coconut, rice, pea, hemp, oat, flax, chufa
IgE versus non-IgE-mediated food allergy	Children with non-IgE-mediated food allergy more likely to react to soy than those with IgE-mediated food allergy

Milk alternatives

Infant formula

A number of guideline articles regarding the management of cow's milk allergy [47–49,50²²,51] have been published, covering IgE to non-IgE-mediated cow's milk allergy.

A large range of infant formula based on amino acids, extensively hydrolyzed whey and casein, hydrolyzed rice-based formula, and soy formula are available though availability varies between countries. Meyer *et al.* [52²³] summarized the available evidence on amino acid-based formulas versus extensively hydrolyzed formulas (Table 1).

The use of maternal allergen avoidance while breastfeeding is still very controversial and a European Academy of Allergy and Clinical Immunology Task Force concludes that in the case of non-IgE-mediated cow's milk allergy, it is currently unclear how often cow's milk allergy occurs in exclusively breastfed infants. Excluding cow's milk from the mother's diet has nutritional implications and should ideally be done under dietetic supervision [53²⁴].

Although not widely used in all countries, data show that both partially and extensively hydrolyzed rice formulas are well tolerated improve clinical symptoms of cow's milk allergy in more than 90% of infants and children and support adequate growth [54,55]. It also does not pose the concern

of inorganic arsenic levels as commercially available rice alternatives [56].

Soy formula is often used in children with cow's milk allergy and despite concerns about possible coexisting allergies a recent review found a very low prevalence (around 10%) of soy allergy in those with cow's milk allergy [57]. The World Allergy Organization recommends that commercially available milks or beverages based should not be used in children under two years. The decision to use these beverages should ideally be preceded by a nutrition assessment [58²⁵]. It is suggested that those with cow's milk allergy should also avoid ewe's milk and goat's milk because of homology or proteins [51].

Cow's milk avoidance

Precision regarding tolerance level

Patients suffering from cow's milk allergy need avoidance advice to ensure patient safety but also to protect from over restriction [59].

Patients need to be instructed on the food allergen-labeling regulations in their particular country and which terms indicate the presence of milk in a product.

Precautionary allergen labeling (PAL) is not regulated and used voluntarily on food products. It is important to understand that not all food labels

with such a statement will contain milk or in amounts large enough to cause an allergic reaction. Equally so, many products that do not contain any PAL may be contaminated with milk, but the clinical implication of consuming these products are unclear, but possibly up to 68% of milk allergic cases [60]. Food produced by smaller factories, candies (chocolate) and cookies were more likely to be contaminated with milk than other products [61]. The eliciting dose for an allergic reaction in 1% of the population estimated for the following were 0.1 mg for cow's milk (0.33 ml of milk) but current food processing and labeling does not allow those with milk allergies to estimate the level of contamination or in many cases the amount of milk protein in a food product [62].

Baked milk

Incorporating baked milk products can decrease the stress of strict avoidance [63], though there is an increasing need for healthier and culturally appropriate (Table 2).

Determining tolerance to baked milk in a safe manner is a continuing debate in the allergy world. The UK guidelines from the British Society of Allergy and Clinical immunology suggest that graded introduction of baked milk at home may be safe in those with mild symptoms on accidental ingestion, no reaction in the past six months and a significant reduction in skin prick test size or specific IgE levels [49]. From a US point of view, Nowak-Wegrzyn *et al.* [64] warn against the possibility of serious reactions and that introducing baked milk at home may pose a risk. There is some debate as to whether continued intake of baked milk products can accelerate tolerance development to unheated milk. A systematic review conducted in 2017 concluded that there is little evidence to suggest that the intake of baked cow's milk results in more patients outgrowing their cow's milk allergy [65]. However, a recent randomized trial demonstrated that baked milk intake can accelerate tolerance to unheated milk after one year of consumption [66].

NUTRITIONAL MANAGEMENT

Challenges posed by nutritional issues

Growth

Cow's milk contributes significantly to macro and micronutrient intake in children. Regular nutritional assessment and monitoring of growth (height and weight) on appropriate growth charts is therefore essential [67,68].

It is important to realize that food allergies do not just represent underweight but that both over and undernutrition in this group of children have been reported [69–71].

Vitamin and mineral supplementation

Cow's milk allergic children are at a higher risk of developing nutrient deficiencies for a wide range of micronutrients, such as iodine, calcium, and vitamin D [72–79]. In children with cow's milk allergy, the nutrition consultation should include a nutritional balanced diet, breast milk supply, the use of a suitable milk-free formula/beverage or multivitamin/mineral supplementation [73]. If breast-feeding women exclude milk from their diet, intake of these nutrients should be addressed as well [80].

Feeding skills

Feeding difficulties or selective eating has not been well characterized in children with IgE-mediated cow's milk allergy, although clinical experience suggests that it does exist and it has been reported in non-IgE-mediated food allergies [73,81,82]. It is important that the allergy specialist dietitian assesses feeding and texture milestones during the allergy assessment [83,84].

Cow's milk allergy and the gut microbiota

Knowledge regarding the gut microbiota, how it is affected by nutritional intake and the role it may play in tolerance development is important [85]. The microbiome plays an important role in

Table 2. Baked milk-containing foods

Established as baked milk-containing foods	Could be considered as baked milk-containing foods but controversial
Cookies/biscuits	Cookies/biscuits with chocolate chips
Crackers (no cheese crackers)	Cheese crackers (used as part of recipe not sprayed)
Muffins/cup cakes	Muffins/cupcakes containing fruit/vegetables
Bread containing milk	Bread containing fruit
Commercial pancakes and waffles	Homemade pancakes and waffles – cooked well
Chocolate chip free brownies – baked very thin and like a crisp/chip	Brownies with nuts and chocolate chip

maintaining the mucosal barrier as both a physical and immunological barrier and in regulating the immune system via its metabolites [86–88]. Microbiota diversity is measured through different indices: α diversity measures the species diversity within the community of a given ecosystem (e.g., diversity within a single sample), whereas β diversity shows the difference between microbial communities from different environments (e.g., diversity between samples). Evidence suggests that the microbiota of children with cow's milk allergy has lower gut microbiota diversity and an elevated Enterobacteriaceae to Bacteroidaceae in early infancy compared to healthy children [89] suggesting that low gut microbiota diversity in early infancy may contribute to the development of food allergy, including CMA.

Increasing the diversity of food intake in infancy has been associated with an increased diversity of the gut microbiota, which in turn seems to be associated with reduced allergy outcomes [90]. There are however a number of factors that may lead to reduced diversity in the diet of child with food allergy such as food restriction and feeding difficulties. There is to date, no study assessing the diet diversity or microbial diversity in children with cow's milk allergy.

Dietary treatment strategies and tolerance development

The particular role of probiotics, prebiotics, and synbiotics in the management and tolerance development in IgE-mediated cow's milk allergy has not been widely studied and there is currently no evidence to recommend these supplements for management and tolerance development in individuals with cow's milk allergy [91].

Canani *et al.* [92,93] showed that using extensively hydrolyzed formula with *Lactobacillus rhamnosus* GG (LGG) improved tolerance development to cow's milk compared with amino acid formulas and extensively hydrolyzed formulas without lactobacillus LGG. Further mouse model work supported the clinical outcomes in humans showing that LGG, added to extensively hydrolyzed formula, significantly enhanced interleukin 13, and anti- β -lactoglobulin IgE production. This may be explained as LGG is a butyrate producer and butyrate, in turn, may affect expression of genes involved in the allergic pathway [94]. Beyond the gut modulation by pre/pro/synbiotics, increasing evidence also suggests the presence or absence of some peptides *per se* might play a role in tolerance induction. As a consequence, the tolerogenic potential of hydrolyzed formulas is currently under investigation [95,96].

Oral immunotherapy

Specific allergen immunotherapy is potentially a curative therapy for IgE-mediated food allergy [97].

Oral immunotherapy (OIT) represents a treatment strategy in patients with severe and persistent IgE-mediated CMA who did not achieve spontaneous tolerance [98]. The most recent evidence confirmed the increase of the tolerated threshold dose in the treated groups, which requires a daily consumption of milk [99]. Baseline characteristics and early treatment reactivity strongly predicted the maintenance of the high-tolerated dose over time and the risk of anaphylaxis.

Several approaches have been recently developed to reduce the occurrence of adverse reactions during milk OIT.

Through a peptide microarray and bioinformatics approach, a combination of different binding peptides from cow's milk caseins and β -lactoglobulin has been proposed as a predictor of safety and efficacy of cow's milk oral immunotherapy before starting the treatment [100].

The goal now is to develop hypoallergenic molecules that contain T-cell epitopes but lack cow's milk specific IgE epitopes. These molecules will maintain their immunogenic and tolerogenic properties without the potential of allergic reactions, making OIT safer. A hypoallergenic β -lactoglobulin has been demonstrated effective *in vitro* as a potential candidate for safer oral immunotherapy [101].

CONCLUSION

The development of omics sciences has led to an important improvement of precision medicine in food allergy. The patient data yielded from the most recent omics technologies allows the practitioner to make a more precise diagnosis, differentiate between CMA subtypes and to predict the persistence of CMA.

The aim of precision medicine is to obtain patient information that can be used to identify the optimal treatment choice for that patient. A patient centered approach, keeping the patient involved in the decision making process is also important. (<https://erj.ersjournals.com/content/50/4/1700391>). Along with the bulk of data derived from recent technologies, data obtained from patient clinical history can be translated into precise patient-tailored interventions in clinical practice. In a near future, the identification of biomarkers will improve the diagnostic accuracy and will also predict patient responsiveness to the treatments. The successful implementation of precision medicine will however depend on understanding and utilizing the increasing amount of data effectively.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

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- of special interest
- of outstanding interest

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