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The Effect on the Immunology Laboratory of the Expansion in Complement Therapeutics

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Abstract:

The approval in the US and Europe of Eculizumab in 2007 marked a change in complement therapeutics, and with it the landscape for complement testing in the clinical immunology laboratory changed. The change had begun even before that when C1-Inhibitor preparations were approved in the 1980s in Europe. There are now two classes of approved drugs that may impact the immunology laboratory, with two dozen more with novel modalities and potential indications that are in various stages of development. Every pathway and about every component of complement has been targeted by these drug development programs, and the modalities of the drugs in development are diverse. These developments will likely result in more laboratories offering more complement testing, so this review looks forward to some of those possible changes in testing.

Keywords: Complement, Therapeutics, Diagnostics, eculizumab, CH50

Introduction:

Complement was recognized for its important role in innate humoral immunity as early as the 1900s, but only in the recent past have we seen the advent of drugs targeting complement [1]. This development was propelled by recognition of the fact that the roles and effects of complement extend far beyond just infection control [2, 3]. There are currently two points in the complement system that are targeted by approved pharmaceuticals, but there are therapeutics in development that target just about every component of complement [4]. The modalities and modes of action are almost as varied as the components targeted [5, 6]. This adds to the complexity of the requests coming to the clinical immunology laboratory around complement testing. Running a C3 and C4 level, and possibly a version of the total complement function assay, may have been sufficient in the past, but as these new therapeutics evolve immunology laboratories face increased calls to do a greater variety of complement testing with accelerated delivery deadlines.

The first therapeutic specifically targeting the complement pathway was Eculizumab (trade name Soliris®) which was approved both in the US and in Europe in 2007 for the treatment of paroxysmal nocturnal hemoglobinuria (PNH) [7]. Before that C1-Inhibitor (C1-INH) was utilized in Europe and other countries as early as 1980s; the HIV epidemic slowed approval in the US. The FDA approved Soliris® then approved Berinert® and Cinryze® in 2008, with approval for Kalbitor® coming in 2009, all for Hereditary Angioedema (HAE) treatment [8-10]. Soliris® was approved in 2011 for a second indication, atypical Hemolytic Uremic Syndrome (aHUS). That same year Firazyr® was approved for HAE; Ruconest® followed in 2014.[11]. Most recently Soliris® was approved for Myasthenia Gravis (MG) and another HAE drug HAEGARDA® was approved in 2017 [12, 13]. These seven approved drugs fall into two areas of inhibition: the HAE drugs' impact is early in the classical and lectin pathways, and Soliris® inhibition is in the terminal pathway. At this point almost every component of the complement cascade is being targeted [6]. This discussion is not intended to be a review of the specific therapeutics in development, as that has been done elsewhere [6, 14, 15]. Instead, we will focus on where in the cascade therapeutics are being targeted and how that is likely to affect clinical immunology laboratory testing. This paper will step through the pathways of complement individually, alongside the current and likely future testing requirements. As we discuss each pathway we will delve into how laboratory testing in complement will be impacted not only by the important step of diagnostic testing, but by the need for testing to follow compliance, to quantify drug levels and to verify the efficacy of the level of drug administration. There is also the strong possibility that these drugs will interfere with testing in a way that has not been an issue for complement testing before. While the disorders and diseases currently

targeted by the approved therapeutics are aimed at the treatment of rare or ultra-rare diseases, drugs in the pipeline are focusing on some far more common diseases, including ANCA-associated vasculitis and age-related macular degeneration, which in turn will change the landscape for the laboratory and make the call for this testing hard to escape. It is not clear when the next wave of complement therapeutics will reach approval, but it is prudent to be aware of the potential impact.

Classical & Lectin Pathways of Complement:

Though the classical and lectin pathways of complement each has its own recognition molecules and initial cleavage enzymes, they share the subsequent components and the same C3 convertase, as well as sharing at least one control protein C1-Inhibitor [16]. For these reasons the two pathways will be considered together. C1-Inhibitor, named for its ability to block the activity of the C1 enzyme C1s, also controls a number of other serine proteases, including the MASPs of the lectin pathway. Importantly for HAE and the drugs approved for its treatment, C1-INH also inhibits many of the serine proteases of the contact pathway including Factor IXa, plasmin, kallikrein and high molecular weight kininogen [13, 17, 18]. In the immunology laboratory current HAE tests involve measuring the level of C1-INH or the failure of C1-INH to control complement. It is actually the ability of C1-INH to inhibit the coagulation pathway that is important in this disease, but the measure of the control of complement is the common test and is accepted for diagnosis [19]. The current requirement from the laboratory around HAE testing is generally limited to the area of diagnosis [19-22]. Testing for drug level has not been an imperative, but as three of the approved therapeutics are C1-INH replacements, measurement of C1-INH level as it is performed for diagnosis, could be utilized to follow drug levels, though the level measured would not be simply the drug level. If a patient is on one of these replacement therapies the level of C1-INH measured in a laboratory will be a total of their endogenous level of C1-INH plus the level of drug. The newer modalities, as represented by the Kalbitor and Firazyr, may call for testing more appropriate for a coagulation laboratory because these two drugs target the contact pathway and not complement.

More specific to complement activity are a number of drugs in development that are aimed at other aspects of the classical and lectin pathway. C1q is the key recognition molecule of the classical pathway, but it has also been shown to be important in neuronal development potentially through driving synaptic pruning [23-25]. Because of this activity C1q is targeted by at least one drug in development [26]. C1q levels have been measured in specialized laboratories for immunodeficiency testing and also as a key differentiator between the hereditary and acquired forms of HAE, so this assay has the potential to be expanded to increase the efficacy of this drug.[13, 17, 19]. Depending on the

drug modality, the C1q level may change. If the drug is an antibody-based blockade, the C1q level itself may not be affected, so it may be that measuring the function of C1q, assessed by measuring CH50 as a secondary marker, will be required. If C1q inhibition is effective, the CH50 would decrease, however it will be important to understand how and where the drug is administered. If administration is local, either by an intrathecal or intraocular injection, then the serum level of CH50 activity would not be expected to reflect the local inhibition.

Another component of the C1 complex C1s is also being targeted[6]. The testing for the efficacy of the C1s therapeutic could be expected to largely mirror that of C1q, by using a test for a decrease in CH50 activity. The cognate enzymes of the lectin pathway, the MASPs, too are being targeted for therapeutic drug development [27]. The assays currently available for testing the lectin pathway are more limited than for the other pathway of complement. This is true for commercial tests and for lab tests developed in specialized complement laboratories. Lectin assays are currently limited to testing for the recognition molecule MBL, and there is one function test available[21]. Unfortunately, there are fewer clinical assays for the other recognize molecules of the lectin pathway, the ficolins and collectins. The MBL level is unlikely to be altered by inhibition of the MASPs but the lectin pathway function may be decreased. While the lectin pathway is undoubtedly a very ancient pathway of complement, it is the one that was more recently identified, therefore is less well understood. There are indications of greater redundancy in the lectin pathway, so it will be interesting to see if clinical inhibition of one of the MASPs but not the other MASPs inhibits lectin pathway function similarly in all patients. This testing will also be complicated by the fact that deficiency in MBL is quite common, reaching 5 to 10 percent of the Caucasian populations [28-30]. For these people there will be no lectin function even prior to treatment, so it would be impossible to follow a decrease in response to treatment. Alternatively, it may be possible to test components of the alternative pathway to investigate the inhibition of the MASPs. There is mounting evidence that MASP3 is involved in cleavage of pro-Factor D to Factor D, presenting another potential function and for the laboratory another avenue for testing [31-34].

Stepping down the pathways, C2 is the next current target for a complement therapeutic development [6]. As C2 is shared by the classical and lectin pathways, an inhibition of C2 would be expected to lead to a decrease in both CH50 and lectin pathway function assessments. Once again, depending on the modality of the drug, the C2 level may not be expected to decrease. With the possible exception of inhibition of MASP3, therapeutics aimed at the classical or lectin pathway may not alter testing of the alternative pathway, leaving measurement of AH50 alternative pathway function normal.

Levels of C4 may be expected to normalize if they were decreased, as the therapeutics are largely aimed at inhibition of function which would reduce cleavage and loss of C4.

Alternative Pathway of Complement:

A lack of proper control, or an overactive alternative pathway of complement, has been implicated in a number of diseases [35-37]. This is likely due in part to the surveillance nature of the alternative pathway as well as its role as an amplification loop for activation that starts in the classical or lectin pathways[16]. The alternative pathway exists essentially in a perpetually primed state, with a low level of tickover activation occurring all the time [38]. In addition, it is believed that the amplification afforded by the alternative pathway is responsible for up to 80% of all the complement activation that occurs [16, 36, 39-41]. Therefore, control of the alternative pathway is critical, but development of specific therapeutic inhibitors of the pathway are not as numerous as development programs for inhibitors of the terminal pathway. The alternative pathway was first described as only an amplification loop; some in the field contend it should be returned to this status [42], but there are also strong advocates and evidence that the alternative pathway has a larger role.

The development of alternative pathway therapeutics has also been set back by the poor Phase 3 results released by Genentech and Roche for Lampalizumab [42]. This antibody-based drug was designed to inhibit the function of Factor D by inhibiting substrate binding to treat age-related macular degeneration (AMD). Unfortunately the drug missed the primary endpoints of the trial, but there was other data from the trial that is still being reviewed and may give some insight into the role of complement inhibition. There is growing belief that better patient stratification or intervention earlier in disease may help lead to a better response rate, two factors that again may rely on complement testing. With these results, the next steps for this drug and how the setback will affect drug development aimed at this inhibition of complement in ocular disease is still to unfold[43]. This has not stopped others from working to target Factor D by different modalities that directly target function.

The cleavage target of Factor D, Factor B is also the target of a therapeutic program[6]. Testing for inhibition of Factor D or Factor B could potentially be achieved by measuring alternative pathway function AH50 which would be expected to decrease while the classical pathway function would remain normal. It may be useful to also measure the level of Factor B as the modality of this therapeutic is one that would lead to a decrease in Factor B levels, systemically, as it is an interfering RNA-based drug.

Central Connection Point C3:

All three activation pathways are connected to the terminal pathway via C3, making C3 a key point in the complement cascade [15, 44, 45]. C3 is also important because of its roles as a source of C3a, one of two anaphylatoxins of complement. C3 is also a key source of the strong opsonizing C3 fragments that are produced during activation. A sign of this importance of complement is the circulating concentration of C3 at 1 to 1.5 mg/mL in serum[22]. For these reasons, there has been some reluctance to target C3 inhibition for fear of reducing these important functions. Still, there are a number of therapeutic programs targeting C3, with at least one having had positive Phase 2 data [15, 46]. With the current complement inhibitory therapeutics, vaccinations are required against the pathogens that are known to affect complement-deficient individuals. Increased vigilance and prophylactic antibiotics are recommended [14]. This would very likely be true for C3 inhibitor therapeutics, so even more stringent vigilance may be required. At this point C3 levels are one of the most common complement tests, but once again a decrease in C3 level may not be measured if the drug is an antibody or small molecule-based inhibitor of C3. Depending on the binding sites of the therapeutic antibody and testing antibody, nephelometric or turbidity measurements during drug administration could be effected. That is to say theoretically the therapeutic and measurement antibodies could compete for binding or on the other side, if the two antibodies have different binding sites, the size and light scattering potential would be increased with the addition of therapeutic antibody bound to the C3. For conditions that lead to decreases in C3, such as C3 glomerulopathy, treatment may result in a normalization of the C3 levels due to the presence of the therapeutic antibody in the measurement or to reduced cleavage, something that may be hard to differentiate. In contrast, C3 function would be expected to decrease with the binding and blockade by the therapeutic antibody. A blockade of C3 would be expected to be reflected in any of the total pathway function measurements, CH50 or AH50 of lectin pathway function, as C3 is a shared component of all three pathways. Importantly, inhibition of C3 would not be expected to affect any laboratory measurements that are restricted to the early points of the individual activation pathways. For example, the level of C4 or Bb measured for a patient prior to treatment would not be expected to change during treatment with a C3 inhibitor. That said, it will be interesting to see as a treatment proceeds if there are any compensatory or feedback mechanisms that lead to changes upstream of a point of inhibition. While such interference is still just a theoretical possibility but will need to be considered and tested should these treatments be brought to market.

In addition to measuring total function, it may also be useful for an immunology laboratory to measure the level of C3 fragment deposition on cells [47, 48]. This has been done before by measuring

the level of C3d on erythrocyte, for a test directed at investigating lupus flares. Similarly, measurement of C3d deposition by flow cytometry may be of use to determine the level of inhibition of C3 cleavage achieved by a complement therapeutic. This is particularly attractive for inhibition of C3 as the circulating C3 fragments are cleared relatively quickly, while C3 opsonins are covalently bound to surfaces and will persist longer and also have direct relevance to the potential modes of action with the potential for adverse responses related to a loss of complement immune complex clearance or infection risk [49].

Terminal Pathway of Complement:

The first complement therapeutic approved by the USFDA targeted the terminal pathway. Eculizumab (trademarked Soliris®), an antibody-based therapeutic that binds C5 and blocks cleavage, was approved for the treatment of the ultra-rare condition paroxysmal nocturnal hemoglobinuria (PNH) in 2007. Due to a somatic mutation, these patients lose the appropriate complement inhibitors on the surface of their cells through the loss of the GPI anchoring capacity [50]. These surface markers, CD59 and CD55, are key parts of the complement system's way of telling self from non-self. With the loss of these components the patient's own red blood cells are insufficiently protected from complement lysis. This leads to the characteristic dark urine, thrombosis and, prior to the advent of Soliris®, a shortened life expectancy. As mentioned, Soliris® has since been approved for two additional indications, but for all three the mechanism of direct action of Soliris® is the same. Therefore, the testing in the laboratory will be largely similar post diagnosis. For PNH, the primary diagnostic testing method is to perform flow cytometry to diagnose the loss of the CD59 and CD55[51]. For aHUS, diagnosis is more complicated, involving first ruling out thrombotic thrombocytopenic purpura (TTP) by testing for loss of function of ADAMTS13. Once that is ruled out, testing is set to look for abnormalities with the complement system. Making this distinction between TTP and aHUS is important as the treatment for one would not be effective for the other. Currently, the testing for aHUS is suggested to be largely molecular testing for genetic abnormalities in a handful of complement genes. However, this testing is too often not definitive as a number of patients will not demonstrate any known genetic abnormalities. Even for those for whom a genetic mutation in a complement gene is found there is still a fair number that will be returned as being an abnormality of unknown significance [52-54]. Protein level complement testing has the potential to be faster and cheaper for diagnosis than the genetic testing, and such testing may determine if there is a phenotypic outcome for the genetic findings of unknown significance. In fact the protein level testing is currently being used in some centers to aid in diagnosis, but is more commonly

used to follow patients on Soliris®. That utility has prompted interest in clinical immunology laboratories in developing additional testing and better standards for that testing. Measurements of classical pathway function (e.g. CH50) have been the most utilized, with measurement of the level of the soluble form of the membrane attack complex (sC5b-9, TTC and less frequently sMAC) finding growing utility.[55, 56]. Measurements of both analytes are being used to determine if complement has been sufficiently blocked. This is of interest both from a treatment perspective and a cost perspective. Soliris® is a very expensive medication, expensive enough that many European health services and U.S. payers have had to look closely at when or even whether to cover Eculizumab treatment [57]. Obviously, there is a drive not to use more of it nor use it longer than is necessary to properly control complement in these patients. When testing function for these patients it is important to understand the limitations of the assay being utilized. Historically, these assays were used mainly as a screen for primary immune-deficiencies where degrees of low versus very low were unnecessary. With these costly medications and the dire conditions being treated, it is important to know the degree of inhibition, thus the low levels of the assay are the most important for this testing. There is published literature indicating that the hemolytic or ELISA methods may be preferred for this testing over the higher throughput liposomal methods of testing total complement function [58]. Table 1 shows the expected effects of these treatments on a panel of complement tests. Complement levels, from function to activation fragments, can vary from individual to individual, so it is ideal to have pretreatment levels on the patient and to then be able to follow the change in these tests over the time of treatment.

With the success and utility of Soliris®, it is not surprising that there are at least ten other therapeutics in development that also target C5, the majority of them also antibody-based [6]. For these drugs, measurement of C5 level would not be useful as the antibodies would not be expected to change the C5 level and may even interfere with standard measurements. However, there are also oligonucleotide-based therapeutics in development. These are designed to alter the transcription of C5, in which case the C5 level would be expected to decrease, so C5 measurement may be useful to follow efficacy of these drugs. For all, measurement of C5 function or the split product of C5, C5a, may seem attractive particularly as C5a is itself an anaphylatoxin with many strong pro-inflammatory properties. However, C5a is cleared very quickly [59]. The soluble form of the membrane attack complex, known as sC5b-9 or TCC, persists much longer, therefore is an attractive alternative. Cleavage of C5 is the starting point for formation of this complex and would be expected to reflect the level of such cleavage and expected to decrease with treatment. While the analyte is increasingly being used to follow Soliris® patients, the full utility of the measurements is still being determined.

In addition to targeting C5, at least one therapeutic program is aiming at the C5 anaphylactic split product C5a itself. This development program is in the early stages, but may represent a challenge for the clinical immunology laboratory. As the therapeutic is antibody in nature, a change in C5a level may not be expected and functional assays for C5a are not yet available. This may be a case where clinical outcomes take precedence over laboratory testing, however nothing spurs laboratory assay development like an unmet clinical need.

Complement Regulators and Receptors:

One regulator of complement, C1-INH, has already been discussed, but Factor H is also being looked at for complement therapeutics. Polymorphisms in Factor H that were found with diseases, including aHUS and age-related macular degeneration, have helped to drive the interest in harnessing the control ability of this regulator [36, 60-63]. Factor H has been implicated in controlling both the alternative and terminal pathways of complement. In addition, Factor H is known to be able to control complement both at cellular surfaces and in circulation. There is good evidence that the control on surfaces, as opposed to the fluid phase, may also reside in a different area of the molecule, making it a possibility to therapeutically target the level of control of one versus the other. While the development of a Factor H-based therapeutic is in the early stage, it would be expected to affect a number of complement assays. If exogenous Factor H-like control is achieved, it would be expected to lead to a decrease in the functional complement mostly in the alternative pathway assays. For functional assessment of the classical or lectin pathway, any decrease would come from inhibition of the terminal pathway and any potential contribution from the amplification loop. The alternative pathway would be expected to exhibit greater control imposed by a Factor H-based therapeutic leading to a low AH50. Inhibition of the alternative pathway components would be expected with a decrease in Bb or Ba levels.

In addition to looking at the regulators of complement, at least one program is targeting one of the receptors that transduce the downstream effects of complement activation. Specifically the C5aR1 is being targeted by a small molecule antagonist. As it is a small molecule, the testing for drug level would not be performed in an immunology laboratory, and there are currently no clinical assays designed to look specifically at the downstream effect of C5aR1 engagement.

Considerations for Testing Interference:

Testing complement is not simple because it is prone to many pre-analytic and analytic problems. These issues have limited the utility and adoption of complement testing and anyone utilizing

this testing should be aware of the following points. Complement can continue to undergo activation after the draw, even when the serum or plasma is stored at -20°C [64-66]. Even more important is collecting the correct specimen type for the test to be performed. Specifically, EDTA plasma is necessary for accurate measurement of the activation fragments of complement, as EDTA inhibits *ex vivo* complement activation, which can occur at room temperature, at -20°C and especially during the clotting process. This *ex vivo* activation of complement can lead to artificially decreased measurement of complement function and artefactual increase in the activation markers. These key issues are being addressed by a committee within the International Complement Society which welcomes laboratories interested in joining those efforts. The group has already expanded the external quality assessment programs available for complement testing.

Beyond the basic issues around complement testing there are some specific points that should be considered in the area of complement therapeutics. When looking at measuring the effectiveness of a therapeutic complement inhibitor, it is important to keep in mind where in the cascade the inhibitor acts. The elements of the cascade that are above the point of action or are in another pathway would not necessarily be affected by the drug in question. That is, an inhibitor of C5 would not be expected to change the level of C3, just as an inhibitor of C2 would not change the level of activation or function of the alternative pathway. This can be valuable in a couple ways. For one, by testing upstream of the inhibition it may be possible to determine if the underlying complement activation has been reduced. Depending on the condition being treated it may be important to know if continued inhibition is necessary or if the underlying activation has resolved. It may occur that downstream inhibition of complement may lead to a compensatory or feedback inhibition that in turn may lead to a decrease of the upstream activation. It has been documented that C5 inhibition by Eculizumab can, for certain conditions, also correlate with a normalization of C3 [55]. It is important to note these pathway placements and distinctions so that conclusions are not incorrect, for example considering a normal C3 level as an indication that C5 blockade is sufficient.

With complement therapeutics it is also important to keep in mind the modality of action of the drug. Most of the drugs in development for complement modulation are antibody-based blockers and this type of drug has the potential to alter the accuracy of some of the more common methods for measuring complement protein levels by altering the equilibrium between the antigen to be measured and the antibody used to do the measurement in nephelometry, turbidity or radial immunodiffusion assays, all equilibrium-based measurements. Testing for such interference may need to be added the laboratory method validations.

Another factor to consider, particularly when utilizing functional complement assays, is the role that complement consumption can have in a low functional assay. If there is strong complement activation, due to either activation from the underlying disease or a failure to control the pathways, this will lead to lower levels of the functional forms of the complement components which in turn will decrease the ability of complement to function in laboratory analysis. Therefore, for disorders where there is substantial complement activation, such as in aHUS, it may be hard to distinguish low CH50 values due to disease activity from low function due to effective inhibition by the drug. In those cases it may be useful to also measure complement activation fragments such as C3a or the complex sC5b-9 to see if they are elevated due to consumption or decreased which would indicate inhibition is likely.

Conclusions:

The rapid increase in therapeutics targeting complement is already putting pressure on immunology laboratories. This pressure includes the demand for more complement analyses, as well as the need to do that testing rapidly. The clinical immunologist may be increasingly called upon to evaluate what testing will be useful for which new therapeutic and for which disorder. With all the aforementioned complications, complement is a cascade of enzymes and proenzymes, something that coagulation laboratories have been adept at handling for years so clearly the challenge can be met. As more therapeutics are approved and are in use by patients, it is possible testing will be needed to determine the best drug for an individual patient. Individual diversity in complement genetics and component levels can mean that a different drug targeted at different part of the complement cascade may be better for an individual patient, even among those coping with the same disease [67]. In this way the clinical immunology laboratory may be called upon to perform complement testing to aid in the administration of precision medicine.

Table 1. Expected Complement Testing Results – Based on Therapeutic Targets and in Comparison to Pre-treatment State

Target	Location On Figure 1	CH50	AH50	C3	C4	C3a	Bb or Ba	sC5b-9
C1q Inhibition	1	Decreased	No change	May Normalize if decreased	May Normalize if decreased	May Normalize if increased	No change ¹	May Normalize if increased
C1s Inhibition	2	Decreased	No change	No change	No change	No change	No change ¹	No change ¹
C1-INH Replacement	3	Normal	No change	Normal	Normalize	Normal	No change ¹	No change ¹
MASP2/MASP3 Inhibition	4	Normal	Normal	May Normalize if decreased	May Normalize if decreased	May Normalize if increased	No change ¹	May Normalize if increased
C2 Inhibition	5	Decreased	No change	No change	No change	No change	No change ¹	May Normalize if increased
C3 Cleavage Blocking	6	Decreased	Decrease	Generally no change	No change	Decrease	Normal If high stay high	May Normalize if increased
Factor B Protein Reduction	7	No change until sufficient to affect amplification	Decrease	May Normalize if decreased	No change	May Normalize if increased	May Normalize if increased	Normalize if was increased due to alternative pathway activation
Factor D Inhibition	8	No change until sufficient to affect amplification	Decrease	May Normalize if decreased	No change	May Normalize if increased	May Normalize if increased	Normalize if was increased due to alternative pathway activation
Factor H Like inhibitors	9	No change until sufficient to affect amplification	Decrease	Normalize if low	No change	May Normalize if increased	May Normalize if increased	Normalize if high

C3 Convertase Inhibition	10	Decrease	Decrease	Normalize if low	No change	May Normalize if increased	May Normalize if increased	Normalize if high
Properdin Inhibition	11	No change until sufficient to affect amplification	Decrease	May Normalize if decreased	No change	May Normalize if increased	May Normalize if increased	Normalize if was increased due to alternative pathway activation
C5 Inhibition/ Cleavage Block	12	Decrease	Decrease	No change	No change	No change	No Change	Decrease
C5a Signal Block	13	No change	No change	No change	No change	No change	No change	No change
C5aR1 Antagonist	NI	No change	No change	No change	No change	No change	No change	No change
C6 Inhibition	14	Decrease	Decrease	No change	No change	No change	No change	Decrease

[†]For the inhibitors of the classical and lectin pathway, it is possible that these will lead to a decrease in alternative pathway activity due to the role of the alternative pathway as an amplification loop. This will depend on the level of activation that is occurring in the individual disease/patient.

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