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Title:

BRIGHT/ARID3A FUNCTION/EXPRESSION AS A MARKER FOR SYSTEMIC LUPUS ERYTHEMATOSUS SEVERITY AND INTENSITY


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(57) Abstract: The present invention involves the identification of elevated Bright/ARID3a levels in the B-cells of systemic lupus erythematosus patients as a marker for impeding disease flare and disease severity. Methods for treating patients so identified are also provided.
DESCRIPTION
BRIGHT/ARID3A FUNCTION/EXPRESSION AS A MARKER FOR SYSTEMIC LUPUS ERYTHEMATOSUS SEVERITY AND INTENSITY

PRIORITY CLAIM

This application claims benefit of priority to U.S. Provisional Application Serial No. 61/828,473, filed May 29, 2013, the entire contents of which are hereby incorporated by reference.

SEQUENCE LISTING

The sequence listing that is contained in the file named "OMRFP0115WO_ST25.txt", which is 20 KB (as measured in Microsoft Windows®) and was created on April 28, 2014, is filed herewith by electronic submission and is incorporated by reference herein.

GOVERNMENT SUPPORT CLAUSE

This invention was made with government support under grant no. R21 #AI90343-2 awarded by the National Institutes Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the fields of autoimmune disease, immunology and molecular biology. More particularly, it concerns Bright/ARID3a activity in the context of systemic lupus erythematosus.

2. Description of Related Art

Systemic lupus erythematosus, or SLE, is an autoimmune disease resulting from breaches in immune tolerance that predominantly affects women. Although this disease may affect as many as 1 in 4000 individuals, the underlying causes are unknown. Environmental factors, hereditary effects and epigenetic variation have all been implicated in the pathogenesis, making it challenging to find a unifying explanation for the complex molecular abnormalities that arise in these patients. SLE is commonly characterized by antinuclear antibody (ANA) production that can develop before the onset of clinical disease, thereby
supporting a multiple hit hypothesis for disease pathogenesis. However, it is a challenging
diagnosis given the overlap of symptoms with other diseases, particularly other autoimmune
disorders.

Treatments for systemic lupus erythematosus (SLE) are complicated by the
extraordinarily heterogeneous nature of disease presentation. Moreover, no current therapies
are successful in all patients, or even in a majority of patients. Furthermore, it is not possible
to faithfully predict which patients will achieve successful remissions with any given
treatment. If SLE patients could be stratified into groups with common underlying
phenotypes, predicting what types of treatments would be most effective would be much
more successful.

SUMMARY OF THE INVENTION

Thus, in accordance with the present invention, there is provided a method of
predicting an auto-antibody flare in a subject with systemic lupus erythematosus (SLE)
comprising (a) obtaining a whole blood sample from a subject; and (b) assessing the level of
Bright/ARID3a in B-cells of said sample, wherein an elevated level of Bright/ARID3a in said
B-cells, as compared to control B-cells from a subject not undergoing a disease flare indicates
that said subject will incur a disease flare. The B-cells may be CD19+ B-cells. The control
B-cells may be from a health subject or a non-flaring SLE subject.

Assessing may comprise immunologic detection of Bright/ARID3a, such as flow
cytometry (FACS), ELISA, RIA or Western blot, or detection of a Bright/ARID3a transcript,
such as employing amplification of Bright/ARID3a mRNA, including RT-PCR. The method
may further comprise performing a SLEDA Index analysis on said subject, and/or further
assessing anti-nuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-
ENA) in a sample from said subject, and/or further comprising taking a medical history of
said subject. The method may further include treating the subject with an SLE therapy if a
flare is predicted.

In another embodiment, there is provided a method of determining the severity of
systemic lupus erythematosus (SLE) in a subject (a) obtaining a whole blood sample from a
subject; and (b) assessing the level of Bright/ARID3a in B-cells of said sample, wherein an
elevated level of Bright/ARID3a in said B-cells, as compared to B-cells from a subject
having moderate SLE, indicates that said subject has severe SLE. The B-cells may be
CD19+ B-cells.
Assessing may comprise immunologic detection of Bright/ARID3a, such as flow cytometry (FACS), ELISA, RIA or Western blot, or detection of a Bright/ARID3a transcript, such as employing amplification of Bright/ARID3a mRNA, including RT-PCR. The method may further comprise performing a SLEDA Index analysis on said subject, and/or further assessing anti-nuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA) in a sample from said subject, and/or further comprising taking a medical history of said subject. The method may further include treating the subject with an SLE therapy if severity is predicted.

In yet another embodiment, there is provided a method of assessing the progression of systemic lupus erythematosus (SLE) in a subject comprising (a) obtaining a whole blood sample from a subject; (b) assessing the level of Bright/ARID3a in B-cells of said first sample; (c) obtaining a second B-cell containing sample from a subject; and (d) assessing the level of Bright/ARID3a in B-cells of said second sample, wherein an elevated level of Bright/ARID3a in B-cells of said second sample, as compared to B-cells from said first sample, indicates that SLE in said subject is progressing. The B-cells may be CD19+ B-cells.

Assessing may comprise immunologic detection of Bright/ARID3a, such as flow cytometry (FACS), ELISA, RIA or Western blot, or detection of a Bright/ARID3a transcript, such as employing amplification of Bright/ARID3a mRNA, including RT-PCR. The method may further comprise performing a SLEDA Index analysis on said subject, and/or further assessing anti-nuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA) in a sample from said subject, and/or further comprising taking a medical history of said subject. The method may further include treating the subject with an SLE therapy if progression is predicted.

In still another embodiment, there is provided a method of assessing the efficacy of a treatment for systemic lupus erythematosus (SLE) in a subject comprising (a) obtaining a first whole blood sample from a subject; (b) assessing the level of Bright/ARID3a in B-cells of said first sample; (c) treating said subject for with an SLE treatment; (d) obtaining a second whole blood sample from a subject; and (e) assessing the level of Bright/ARID3a in B-cells of said second sample, wherein a reduced level of Bright/ARID3a in B-cells of said second sample, as compared to B-cells from said first sample, indicates that said SLE treatment was effective. The B-cells may be CD19+ B-cells.

Assessing may comprise immunologic detection of Bright/ARID3a, such as flow cytometry (FACS), ELISA, RIA or Western blot, or detection of a Bright/ARID3a transcript,
such as employing amplification of Bright/ARID3a mRNA, including RT-PCR. The method may further comprise performing a SLEDA Index analysis on said subject, and/or further assessing anti-nuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA) in a sample from said subject, and/or further comprising taking a medical history of said subject.

In still a further embodiment, there is provided a method of treating a subject with systemic lupus erythematosus (SLE) in a subject (a) obtaining a whole blood sample from a subject; (b) assessing the level of Bright/ARID3a in B-cells of said sample; and (c) treating said subject when said B-cells exhibit an elevated level of Bright/ARID3a as compared to B-cells from a healthy control. The B-cells may be CD19+ B-cells.

Assessing may comprise immunologic detection of Bright/ARID3a, such as flow cytometry (FACS), ELISA, RIA or Western blot, or detection of a Bright/ARID3a transcript, such as employing amplification of Bright/ARID3a mRNA, including RT-PCR. The method may further comprise performing a SLEDA Index analysis on said subject, and/or further assessing anti-nuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA) in a sample from said subject, and/or further comprising taking a medical history of said subject.

An additional embodiment provides a kit comprising (a) one or more reagents for assessing the expression of Bright/ARID3a in a biological sample; and (b) one or more reagents for assessing anti-nuclear antibody (ANA) testing and/or anti-extractable nuclear antigen (anti-ENA) in a biological sample. The kit may further comprise an agent for treating systemic lupus erythematosus, such as a Bright/ARID3a inhibitor, including an interfering RNA, a dominant-negative Bright/ARID3a molecule, a Bright/ARID3a peptide or an expression vector coding therefor. The agent may also be a corticosteroid or an anti-malarial drug.

As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising," the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more. Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.
BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIGS. 1A-D. ARID3a is over-expressed in B lineage cells in a subset of SLE patients. (FIG. 1A) Immunofluorescent staining of ARID3a+ cells in B (CD19+) and T (CD3+) lymphocytes from a representative control (Ctrl 6) and 2 lupus patients (SLE #15, #2) are shown relative to isotype controls. (FIG. 1B) Mononuclear cells from a representative control and 4 SLE patients were assessed by Western blotting. CLO1 is a positive control. Data represent > 6 experiments. (FIG. 1C) Numbers of ARID3a+ B cells/ml are shown for 42 SLE, 6 rheumatoid arthritis (RA) and 18 healthy controls. Open circles indicate patients with numbers of ARID3a+ cells > 2 standard deviations above mean numbers for controls (designated as ARID3aH). (FIG. 1D) The mean SLEDAI score of patients with ARID3aH samples (squares, n=17) was higher than the mean from patients with numbers of ARID3a+ cells within normal range (ARID3aN, circles, n=23). Each symbol represents one SLE patient sample. Statistics were determined by Student’s t test. Means ± SEM are shown.

FIGS 2A-B. ARID3a expressing cells occur in multiple B cell subpopulations in SLE patients. (FIG. 2A) Histograms show ARID3a expression in 6 B cell subsets for ARID3aN, ARID3aH and control samples compared to isotype controls (left panels). Bars were set relative to the isotype controls. (FIG. 2B) Total numbers of ARID3a+ B cells/ml in each B cell subset from 42 SLE, 6 RA and 17 healthy controls were enumerated. Means ± SEM are shown. Mann-Whitney calculated p values are given. Zero values were included in data analyses.

FIG. 3. Numbers of ARID3a+ B cells in individual patients vary with time. Numbers of ARID3a+ CD19+ B cells/ ml of blood from SLE patients subdivided based on total ARID3a+ B cells at their first visit (ARID3aH, n=15 or ARID3aN, n=22) were plotted for each visit. Each symbol represents one patient sample. Red dotted lines show mean numbers of ARID3a+ B cells from healthy controls; black dashed lines show the value used to designate samples as ARID3aH or ARID3aN.
FIGS 4A-B. ARID3a expression did not correlate with CD86 and CD43 activation markers in CD27 B cells. Peripheral blood mononuclear cells were stained for CD27, CD43, CD86 and ARID3a. (FIG. 4A) Mean percentages of CD27+, ARID3a+ and ARID3a– B cells in CD86 and CD43 gates and (FIG. 4B) mean percentages of CD27+, CD86+ and CD86– and CD43+ and CD43– B cells in the ARID3a gate from 42 SLE, 15 controls and 4 RA patient samples are shown.

FIG. 5. Circulating IL-10 levels were increased in SLE patients with increased numbers of ARID3a+ B cells. Plasma levels of TGF-β, IL-17, and IL-10 from ARID3a (7-15) and ARID3a (9-24) samples were measured and calculated relative to standard curves. Each symbol represents a different sample. Means and SEMs are indicated.

FIG. 6. Immunofluorescent gating of peripheral blood B lineage subpopulations. Representative data show surface markers and flow cytometric-gating strategies used to identify individual B lymphocyte subsets. Numbers correspond to common nomenclature for CD19+ B cell subsets defined as: naive IgD+ B cells (IgD+IgM–CD27+), C8 class-switched (IgD+IgM–CD27+), naive (IgD+IgM+CD27+CD38–), marginal zone (MZ) memory (IgD+IgM+CD27+CD38–), memory IgM (IgDTgM+CD27+), class-switched memory (IgD–IgM–CD27+CD38+), double negative (DN) cells (IgDTgM+CD27–CD38–), and plasmablast/plasma cells (IgDTgM–CD27–CD38–).

FIG. 7. Increased numbers of ARID3a+ cells are not due to expansions of specific B cell subsets. CD19+ B cells from healthy control, ARID3a and ARID3a patient samples were stained and B cell subsets were gated as shown in FIG. 6. Each symbol represents one sample. Samples with <1000 total B cells/ml were excluded because B cell subsets numbers were too few for valid enumeration. Means ± SEM are shown. Zero values were included in analyses. Mann-Whitney analyses showed increased ARID3a memory IgM cells versus controls (p= 0.0428).

FIGS. 8A-B. Transitional B cells did not account for increased numbers of ARID3a+ B cells. Quantification of transitional (CD10+) and naive (CD10–) B cells (FIG. 8A) and numbers of ARID3a+ cells (FIG. 8B) within those subsets were determined in peripheral blood of healthy controls, ARID3a patients, and ARID3a patients by flow cytometry. Each symbol represents a different sample. Means and SEMs are indicated.

FIG. 9. Increased SLEDAI scores are correlated with numbers of ARID3a+ CD19+ B cells. Total numbers of ARID3a+ CD19+ B cells from each patient at each visit and the corresponding SLEDAI scores at the respective visits were subjected to
Spearman's rank correlation coefficient analyses to determine their relationship. Rank correlation and p values are shown.

**FIGS. 10A-B. Plasma immunoglobulin levels are higher in ARID3aH SLE samples, but all samples had similar autoreactivities.** (FIG. 10A) Relative plasma IgM and IgG levels from control (n=19), ARID3aH (n=44) and ARID3aN (n=19) samples are shown as averages of duplicates. Each symbol represents one sample. Error bars indicate mean ± SEM. Significance by Student's t-test is indicated by p values. (FIG. 10B) Percentages of plasma from 16 ARID3aH and 26 ARID3aN samples positive for common cross-reactivities are presented.

**DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS**

Multiple murine models for lupus exist. In keeping with the complex regulatory mechanisms that control immune responses, these models may involve disruptions in genes expressed in T or B lymphocytes, or may result from combined defects in genes expressed in a variety of immune regulatory cells (8,9). While each of these models results in ANA production, they all have limitations and differ in the extent to which they mimic the human SLE organ involvement that typically evolves over time within individual patients. The inventor shows here that transgenic mice that overexpress the DNA-binding protein Bright/ARID3a (B cell regulator of immunoglobulin heavy chain transcription/A+T rich interaction domain family protein 3a) in all B lineage cells produced serum ANAs by four weeks of age (10,11). Overexpression also resulted in increased numbers of marginal zone (MZ) B cells which are typically enriched for self-reactive B lymphocytes (11). These data suggest that inappropriate regulation of Bright/ARID3a expression in B lineage cells is sufficient to cause ANA production in these mice.

Because constitutive expression of Bright/ARID3a in B cells of transgenic mice resulted in ANA production, a predisposing occurrence for SLE (12), the inventor asked if SLE patients exhibit increased ARID3a expression in their peripheral blood B lymphocytes. The results show here that nearly half of SLE patients overexpress a DNA-binding protein called ARID3a in peripheral blood B lymphocytes. It therefore the inventor's proposal that division of patients into groups based on ARID3a expression levels will be useful to predict disease progression and response. Overexpression of ARID3a (or Bright as it was first called in the mouse (Webb et al., 1991; Herscher et al., 1995) in B lineage cells of transgenic mice was also associated with autoantibody (antinuclear antigen, ANA) production in 100% of the
mice as early as four weeks of age (Shankar et al., 2007; Oldham et al., 2011). These mice accumulated glomerular immune complexes with time. Furthermore, overexpression of ARID3a in mouse B lineage cells altered the gene expression pattern of the resulting mature follicular B cells so that they resembled marginal zone B cells, a subpopulation of B cells associated with autoantibody production (Oldham et al., 2011). These data strongly suggest that ARID3a overexpression contributes to autoimmunity, possibly by driving the development of mature B cells that produce autoantibodies.

The inventor also showed that ARID3a expression in patients’ B cells was not limited to the B cell subsets that normally express ARID3a, but that it was expressed in some cells at every stage of differentiation. Over the past three years, a longitudinal study of 44 patients was conducted over three separate visits and the numbers of B cells in each subpopulation quantitated, including the numbers of cells within those subpopulations that express ARID3a. The inventor was also provided with SLEDAI scores to measure disease activity in these patients. Increased numbers of ARID3a+ B cells correlated with disease activity on one visit (FIG. 14). More importantly, inclusion of data from multiple visits showed strongly significant correlations between disease activity (as measured by SLEDAI scores) and numbers of ARID3a+ total B cells, ARID3a+ plasmablasts (which are activated to secrete antibody) and ARID3a+ Marginal Zone memory cells (see Table 2). These cells develop from naïve B cell subpopulations, and presumably from those that are ARID3a+. In healthy individuals, ARID3a+ cells are not usually observed in the naïve B cell subpopulation. These cells have not yet begun to secrete antibody.

The inventor proposes that identification of increased numbers of ARID3a+ cells, particularly in the naïve B cell subpopulation, will help identify individuals who may be about to generate an inflammatory response that could correlate with disease activity. In other words, determining ARID3a could help predict disease flares. This would define a new biomarker for disease activity. These and other aspects of the invention are described below.

1. **Bright/ARID3a**

The transcription factor Bright (B cell regulator of IgH transcription) is a member of a growing family of proteins that interact with DNA through a highly conserved A+T-rich interaction domain, or ARID (Herrschter et al., 1995). Currently, Bright is the only member of this family for which target sequences have been identified, and which binds to DNA in a sequence-specific fashion. ARID family proteins include the *Drosophila* proteins Dead ringer and eyelid that play important roles in lineage decisions in the gut and eyelid of the
fruit fly, and are required for embryonic segmentation (Gregory et al., 1996; Treisman et al.,
1997); retinoblastoma binding protein (Rbp1) that interacts with retinoblastoma protein in a
cell cycle-specific fashion (Fattaey et al., 1993); and BDP, a ubiquitously expressed human
protein identified in a two-hybrid screen as a novel protein that also interacts with
retinoblastoma protein (Rb) (Numata et al., 1999). The yeast protein SWI/1 has homology to
Bright, and is a component of a larger protein complex that serves to modulate chromatin
organization in that organism (Peterson and Herskowitz, 1992; Burns and Peterson, 1997).
Likewise, the human SWI-SNF complex contains a 270 kDa protein with non-sequence
specific DNA binding activity that is also a member of the ARID family (Dallas et al., 2000).
Thus, members of this family may participate in lineage decisions, cell cycle control, tumor
suppression and modulation of chromatin. These functions are not mutually exclusive and
may result from overlapping mechanisms.

Sequencing of the human genome identified fifteen members of this family, including
the human Bright ortholog known as ARID3a (Wilsker et al., 2005). ARID family proteins
have diverse functions including, chromatin remodeling, binding to retinoblastoma protein,
regulating X-Y chromosome functions and participation in embryonic development (Wilsker
et al., 2005). Generally, these proteins are components of large protein complexes and are
tightly regulated throughout development. Human ARID3a can bind to E2F in cell lines of
embryonic origin where its over-expression is controversial, as it has been associated with
both tumor suppressor and oncogenic functions (Peeper et al., 2002; Suzuki et al., 1998; Ma
et al., 2003; Fukuyo et al., 2004). Recent studies indicate that Bright activity is regulated
tightly through intracellular partitioning and that it contributes to chromatin accessibility of
the heavy chain enhancer (Kim and Tucker, 2006; Lin et al., 2007). It is likely that
Bright/ARID3a can participate in a wide variety of regulatory functions in both embryonic
and adult tissues because it functions as both a transcription factor and has a role in altering
chromatin accessibility.

Most ARID family proteins are expressed ubiquitously. However, murine Bright is
expressed widely throughout embryonic development, but expression in the adult is largely
limited to the B lymphocyte lineage where its expression is tightly regulated and is restricted
at the mRNA level to the pre-B cell and peanut agglutinin-high germinal center cell
populations (Herrschel et al., 1995; Webb et al., 1991; Webb et al., 1998). Activated splenic
B cells in the mouse can be induced to express Bright after antigen binding, but the protein is
not present in the majority of peripheral IgM+ B cells (Webb et al., 1991; Webb et al., 1998).
Induction of Bright expression in B cell lines or in mature activated B lymphocytes using
lipopolysaccharide or antigen results in upregulation of IgH transcription approximately 3- to 6-fold above basal levels (Herrscher et al., 1995; Webb et al., 1991; Webb et al., 1989). Transcriptional activation is tightly associated with DNA binding sites 5’ of some vH promoters or within the intronic Eμ enhancer.

Bright binding sites associated with the intronic Eμ enhancer also function as matrix-association regions, or MARs, A+T rich regions that have been proposed to organize chromatin into transcriptionally active domains (Herrscher et al. 1995; Webb et al., 1991). NFμNR (nuclear factor μ negative regulator) is another MAR-binding protein complex that binds DNA sequences overlapping Bright binding sites. NFμNR contains the ubiquitously expressed CAAAT displacement protein (CDP/Cut/Cux) (Wang et al., 1999). While non-B cells in the mouse express NFμNR, B lymphocytes generally do not exhibit such protein complexes. These data have led to the hypothesis that Bright and NFμNR play opposing roles in regulating the immunoglobulin locus (Webb et al., 1999). Transfection studies in which Bright and CDP were coexpressed showed repression of Bright (Wang et al., 1999).

Therefore, Bright may activate transcription, directly or indirectly through chromatin remodeling or through more complex interactions with additional proteins. NFμNR may act in opposition to that activity (Wang et al., 1999).

The inventor has shown that Bruton’s tyrosine kinase, or Btk, associates with Bright in activated murine B lymphocytes (Webb et al., 2000). Btk is an X-linked gene that encodes a tyrosine kinase critical for proper development and maintenance of B lymphocytes both in humans and in mice (reviewed in (Conley et al., 1994; Satterthwaite and Witte, 1996). Defects in this enzyme account for 90% of the severe B cell immunodeficiencies in man, and result in X-linked agammaglobulinemia (XLA), an immunodeficiency state characterized by blocks at the pro-B cell stage of development and severely depressed serum antibody levels (Conley et al., 1994). Although Btk is clearly the defective gene product in both human and murine diseases, the molecular mechanisms by which Btk deficiencies result in blocks in B cell development are currently unknown. Of interest, X-linked immunodeficient (xid) mice, the mouse model for XLA, produce a mutated Btk protein that fails to form stable complexes with Bright (Webb et al., 2000). These data suggest that Bright may function as a component of the same signaling pathway(s) important in XLA.

Very little information is available regarding human Bright protein. Therefore, the inventor sought to characterize the human Bright homologue and to determine its expression in B lymphocyte subpopulations. Bright was cloned from a human B cell library and the
sequence was determined to be identical to that published previously as Dril 1 (Kortschak et al., 1998). Although these studies suggested that Dril 1, or human Bright, mRNA was expressed in multiple tissues (Kortschak et al., 1998), protein and DNA binding activity were not investigated. The inventor's data indicate that Bright/Dril 1 mRNA may be expressed in a smaller number of adult tissues than previously thought. Furthermore, these data demonstrate that the human protein effectively binds the Bright prototype sequence motif. Investigation of sorted B cell subpopulations demonstrated that human Bright expression was similar in many ways to expression of the murine homologue; although, Bright mRNA was expressed at slightly earlier stages of normal B cell development in man than in the mouse. On the other hand, expression of Bright protein in human transformed cell lines differed dramatically from that observed in the mouse. Finally, results reveal that human Bright and Btk associate to form DNA-binding complexes, which further involve the Btk substrate TFII-I (Rajaiya et al., 2006).

II. Peptides and Polypeptides

In certain embodiments, the present invention may concerns Bright/ARID3a protein molecules. As used herein, a "protein" or "polypeptide" generally refers, but is not limited to, a protein of greater than about 100 amino acids or the full length endogenous sequence translated form of a gene. A peptide is usually from about 3 to about 100 amino acids. All the "proteinaceous" terms described above may be used interchangeably herein. A human ARID3a polypeptide sequence is provided in SEQ ID NO:2.

Proteins may be produced recombinantly or purified from natural sources. Shorter peptide molecules may be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, e.g., Stewart and Young (1984); Tarn et al. (1983); Merrifield (1986); and Barany and Merrifield (1979), each incorporated herein by reference.

In certain embodiments the size of the at least one proteinaceous molecule may comprise, but is not limited to, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60,
about 61, about 62, about 63, about 64, about 65, about 66, about 67, about 68, about 69,
about 70, about 71, about 72, about 73, about 74, about 75, about 76, about 77, about 78,
about 79, about 80, about 81, about 82, about 83, about 84, about 85, about 86, about 87,
about 88, about 89, about 90, about 91, about 92, about 93, about 94, about 95, about 96,
about 97, about 98, about 99, about 100, about 110, about 120, about 130, about 140, about
150, about 160, about 170, about 180, about 190, about 200, about 210, about 220, about 230,
about 240, about 250, about 275, about 300, about 325, about 350, about 375, about 400,
about 425, about 450, about 475, about 500, about 505, about 525, about 550, about 575 and
593 amino molecule residues, and any range derivable therein.

As used herein, an "amino acid" refers to any amino acid, amino acid derivative or
amino acid mimic as would be known to one of ordinary skill in the art. In certain
embodiments, the residues of the proteinaceous molecule are sequential, without any non-
amino molecule interrupting the sequence of amino molecule residues. In other
embodiments, the sequence may comprise one or more non- amino molecule moieties. In
particular embodiments, the sequence of residues of the proteinaceous molecule may be
interrupted by one or more non-amino molecule moieties.

In certain embodiments, the proteinaceous composition comprises at least one protein,
polypeptide or peptide. In further embodiments, the proteinaceous composition comprises a
biocompatible protein, polypeptide or peptide. As used herein, the term "biocompatible"
refers to a substance which produces no significant untoward effects when applied to, or
administered to, a given organism according to the methods and amounts described herein.
Such untoward or undesirable effects are those such as significant toxicity or adverse
immunological reactions. In preferred embodiments, biocompatible protein, polypeptide or
peptide containing compositions will generally be mammalian proteins or peptides or
synthetic proteins or peptides each essentially free from toxins, pathogens and harmful
immunogens.

Proteinaceous compositions may be made by any technique known to those of skill in
the art, including the expression of proteins, polypeptides or peptides through standard
molecular biological techniques, the isolation of proteinaceous compounds from natural
sources, or the chemical synthesis of proteinaceous materials. The nucleotide and protein,
polypeptide and peptide sequences for various genes have been previously disclosed, and
may be found at computerized databases known to those of ordinary skill in the art. One such
database is the National Center for Biotechnology Information's Genbank and GenPept
databases (world-wide-webe at ncbi.nlm.nih.gov). The coding regions for these known genes
may be amplified and/or expressed using the techniques disclosed herein or as would be
know to those of ordinary skill in the art. Alternatively, various commercial preparations of
proteins, polypeptides and peptides are known to those of skill in the art.

Peptides may also be fused to other proteinaceous compositions, thereby altering or
supplementing their properties. In a particular embodiment, a targeting moiety may be
provided which facilitate cellular transport of the Bright derived peptide or polypeptide. In
particular, sequences such as Tat can provide nuclear localization signals, thereby
transporting peptides into the nucleus.

In certain embodiments, a proteinaceous compound may be purified. Generally,
"purified" will refer to a specific or protein, polypeptide, or peptide composition that has
been subjected to fractionation to remove various other proteins, polypeptides, or peptides,
and which composition substantially retains its activity, as may be assessed, for example, by
the protein assays, as would be known to one of ordinary skill in the art for the specific or
desired protein, polypeptide or peptide.

III. Nucleic Acids

In certain embodiments of the present invention, nucleic acids derived from or
encoding ARID3a are provided. In certain aspects, the nucleic acids may comprise wild-type
or a mutant version of these genes. In particular aspects, the nucleic acid encodes for or
comprises a transcribed nucleic acid. In other aspects, the nucleic acid comprises a nucleic
acid segment of SEQ ID NO:1, or a biologically functional equivalent thereof. In particular
aspects, the nucleic acid encodes a protein, polypeptide, or peptide.

The term "nucleic acid" is well known in the art. A "nucleic acid" as used herein will
generally refer to a molecule (i.e., a strand) of DNA, RNA or a derivative or analog thereof,
comprising a nucleobase. A nucleobase includes, for example, a naturally-occurring purine
or pyrimidine base found in DNA (e.g., an adenine "A," a guanine "G," a thymine "T" or a
cytosine "C") or RNA (e.g., an "A," a "G," an uracil "U" or a "C"). The term "nucleic acid"
embrace the terms "oligonucleotide" and "polynucleotide," each as a subgenus of the term
"nucleic acid." The term "oligonucleotide" refers to a molecule of between about 3 and about
100 nucleobases in length. The term "polynucleotide" refers to at least one molecule of
greater than about 100 nucleobases in length.

These definitions generally refer to a single-stranded molecule, but in specific
embodiments will also encompass an additional strand that is partially, substantially or fully
complementary to the single-stranded molecule. Thus, a nucleic acid may encompass a
double-stranded molecule or a triple-stranded molecule that comprises one or more complementary strand(s) or "complement(s)" of a particular sequence comprising a molecule. As used herein, a single-stranded nucleic acid may be denoted by the prefix "ss," a double-stranded nucleic acid by the prefix "ds," and a triple-stranded nucleic acid by the prefix "ts."

1. Preparation of Nucleic Acids

A nucleic acid may be made by any technique known to one of ordinary skill in the art, such as for example, chemical synthesis, enzymatic production or biological production. Non-limiting examples of a synthetic nucleic acid (e.g., a synthetic oligonucleotide), include a nucleic acid made by in vitro chemically synthesis using phosphotriester, phosphite or phosphoramidite chemistry and solid phase techniques such as described in EP 266 032, incorporated herein by reference, or via deoxynucleoside H-phosphonate intermediates as described by Froehler et al. (1986) and U.S. Patent 5,705,629, each incorporated herein by reference. In the methods of the present invention, one or more oligonucleotide may be used.

Various different mechanisms of oligonucleotide synthesis have been disclosed in for example, U.S. Patents 4,659,774, 4,816,571, 5,141,813, 5,264,566, 4,959,463, 5,428,148, 5,554,744, 5,574,146, 5,602,244, each of which is incorporated herein by reference.

A non-limiting example of an enzymatically produced nucleic acid include one produced by enzymes in amplification reactions such as PCR™ (see for example, U.S. Patent 4,683,202 and U.S. Patent 4,682,195, each incorporated herein by reference), or the synthesis of an oligonucleotide described in U.S. Patent 5,645,897, incorporated herein by reference. A non-limiting example of a biologically produced nucleic acid includes a recombinant nucleic acid produced (i.e., replicated) in a living cell, such as a recombinant DNA vector replicated in bacteria (see for example, Sambrook et al. 2001, incorporated herein by reference).

2. Purification of Nucleic Acids

A nucleic acid may be purified on polyacrylamide gels, cesium chloride centrifugation gradients, or by any other means known to one of ordinary skill in the art (see for example, Sambrook et al, 2001, incorporated herein by reference).

In certain aspect, the present invention concerns a nucleic acid that is an isolated nucleic acid. As used herein, the term "isolated nucleic acid" refers to a nucleic acid molecule (e.g., an RNA or DNA molecule) that has been isolated free of, or is otherwise free of, the bulk of the total genomic and transcribed nucleic acids of one or more cells. In certain
embodiments, "isolated nucleic acid" refers to a nucleic acid that has been isolated free of, or is otherwise free of, bulk of cellular components or in vitro reaction components such as for example, macromolecules such as lipids or proteins, small biological molecules, and the like.

3. **Nucleic Acid Segments**

In certain embodiments, the nucleic acid is a nucleic acid segment. As used herein, the term "nucleic acid segment," are smaller fragments of a nucleic acid, such as for non-limiting example, those that encode only part of ARID3a. Thus, a "nucleic acid segment" may comprise any part of a gene sequence, of from about 2 nucleotides to the full length of ARID3a. In certain embodiments, the nucleic acid segment may be a probe or primer. As used herein, a "probe" generally refers to a nucleic acid used in a detection method or composition. As used herein, a "primer" generally refers to a nucleic acid used in an extension or amplification method or composition.

4. **Nucleic Acid Complements**

The present invention also encompasses a nucleic acid that is complementary to a Bright-encoding nucleic acid. In particular embodiments the invention encompasses a nucleic acid or a nucleic acid segment complementary to the sequence set forth in SEQ ID NO:1. A nucleic acid is a "complement(s)" or is "complementary" to another nucleic acid when it is capable of base-pairing with another nucleic acid according to the standard Watson-Crick, Hoogsteen or reverse Hoogsteen binding complementarity rules. As used herein "another nucleic acid" may refer to a separate molecule or a spatial separated sequence of the same molecule.

As used herein, the term "complementary" or "complement(s)" also refers to a nucleic acid comprising a sequence of consecutive nucleobases or semiconsecutive nucleobases (e.g., one or more nucleobase moieties are not present in the molecule) capable of hybridizing to another nucleic acid strand or duplex even if less than all the nucleobases do not base pair with a counterpart nucleobase. In certain embodiments, a "complementary" nucleic acid comprises a sequence in which about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, to about 100%, and any range derivable therein, of the nucleobase sequence is capable of base-pairing with a single or double stranded nucleic
acid molecule during hybridization. In certain embodiments, the term "complementary" refers to a nucleic acid that may hybridize to another nucleic acid strand or duplex in stringent conditions, as would be understood by one of ordinary skill in the art.

In certain embodiments, a "partly complementary" nucleic acid comprises a sequence that may hybridize in low stringency conditions to a single or double stranded nucleic acid, or contains a sequence in which less than about 70% of the nucleobase sequence is capable of base-pairing with a single or double stranded nucleic acid molecule during hybridization.

5. Hybridization

As used herein, "hybridization," "hybridizes" or "capable of hybridizing" is understood to mean the forming of a double or triple stranded molecule or a molecule with partial double or triple stranded nature. The term "anneal" as used herein is synonymous with "hybridize." The term "hybridization," "hybridize(s)" or "capable of hybridizing" encompasses the terms "stringent condition(s)" or "high stringency" and the terms "low stringency" or "low stringency condition(s)."

As used herein "stringent condition(s)" or "high stringency" are those conditions that allow hybridization between or within one or more nucleic acid strand(s) containing complementary sequence(s), but precludes hybridization of random sequences. Stringent conditions tolerate little, if any, mismatch between a nucleic acid and a target strand. Such conditions are well known to those of ordinary skill in the art, and are preferred for applications requiring high selectivity. Non-limiting applications include isolating a nucleic acid, such as a gene or a nucleic acid segment thereof, or detecting at least one specific mRNA transcript or a nucleic acid segment thereof, and the like.

Stringent conditions may comprise low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C. It is understood that the temperature and ionic strength of a desired stringency are determined in part by the length of the particular nucleic acid(s), the length and nucleobase content of the target sequence(s), the charge composition of the nucleic acid(s), and to the presence or concentration of formamide, tetramethylammonium chloride or other solvent(s) in a hybridization mixture.

It is also understood that these ranges, compositions and conditions for hybridization are mentioned by way of non-limiting examples only, and that the desired stringency for a particular hybridization reaction is often determined empirically by comparison to one or more positive or negative controls. Depending on the application envisioned it is preferred to
employ varying conditions of hybridization to achieve varying degrees of selectivity of a nucleic acid towards a target sequence. In a non-limiting example, identification or isolation of a related target nucleic acid that does not hybridize to a nucleic acid under stringent conditions may be achieved by hybridization at low temperature and/or high ionic strength. Such conditions are termed "low stringency" or "low stringency conditions," and non-limiting examples of low stringency include hybridization performed at about 0.15 M to about 0.9 M NaCl at a temperature range of about 20°C to about 50°C. Of course, it is within the skill of one in the art to further modify the low or high stringency conditions to suit a particular application.

As used herein "wild-type" refers to the naturally occurring sequence of a nucleic acid at a genetic locus in the genome of an organism, or a sequence transcribed or translated from such a nucleic acid. Thus, the term "wild-type" also may refer to an amino acid sequence encoded by a nucleic acid. As a genetic locus may have more than one sequence or alleles in a population of individuals, the term "wild-type" encompasses all such naturally occurring allele(s). As used herein the term "polymorphic" means that variation exists (i.e., two or more alleles exist) at a genetic locus in the individuals of a population. As used herein "mutant" refers to a change in the sequence of a nucleic acid or its encoded protein, polypeptide or peptide that is the result of the hand of man.

The present invention also concerns the isolation or creation of a recombinant construct or a recombinant host cell through the application of recombinant nucleic acid technology known to those of skill in the art or as described herein. A recombinant construct or host cell may comprise a Bright-encoding nucleic acid, and may express a Bright protein, peptide or peptide, or at least one biologically functional equivalent thereof.

Herein certain embodiments, a "gene" refers to a nucleic acid that is transcribed. In certain aspects, the gene includes regulatory sequences involved in transcription, or message production or composition. In particular embodiments, the gene comprises transcribed sequences that encode for a protein, polypeptide or peptide. As will be understood by those in the art, this function term "gene" includes both genomic sequences, RNA or cDNA sequences or smaller engineered nucleic acid segments, including nucleic acid segments of a non-transcribed part of a gene, including but not limited to the non-transcribed promoter or enhancer regions of a gene. Smaller engineered gene nucleic acid segments may express, or may be adapted to express using nucleic acid manipulation technology, proteins, polypeptides, domains, peptides, fusion proteins, mutants and/or such like.
"Isolated substantially away from other coding sequences" means that the gene of interest forms the significant part of the coding region of the nucleic acid, or that the nucleic acid does not contain large portions of naturally-occurring coding nucleic acids, such as large chromosomal fragments, other functional genes, RNA or cDNA coding regions. Of course, this refers to the nucleic acid as originally isolated, and does not exclude genes or coding regions later added to the nucleic acid by the hand of man.

The nucleic acid(s) of the present invention, regardless of the length of the sequence itself, may be combined with other nucleic acid sequences, including but not limited to, promoters, enhancers, polyadenylation signals, restriction enzyme sites, multiple cloning sites, coding segments, and the like, to create one or more nucleic acid construct(s). As used herein, a "nucleic acid construct" is a nucleic acid engineered or altered by the hand of man, and generally comprises one or more nucleic acid sequences organized by the hand of man.

In a non-limiting example, one or more nucleic acid constructs may be prepared that include a contiguous stretch of nucleotides identical to or complementary to SEQ ID NO:1. A nucleic acid construct may be about 3, about 5, about 8, about 10 to about 14, or about 15, about 20, about 30, about 40, about 50, about 100, about 200, about 500, about 1,000, about 2,000, about 3,000, about 5,000, or about 10,000 nucleotides in length, as well as constructs of greater size, up to and including chromosomal sizes (including all intermediate lengths and intermediate ranges), given the advent of nucleic acids constructs such as a yeast artificial chromosome are known to those of ordinary skill in the art. It will be readily understood that "intermediate lengths" and "intermediate ranges," as used herein, means any length or range including or between the quoted values (i.e., all integers including and between such values). Non-limiting examples of intermediate lengths include about 11, about 12, about 13, about 16, about 17, about 18, about 19, etc.; about 21, about 22, about 23, etc.; about 31, about 32, etc.; about 51, about 52, about 53, etc.; about 101, about 102, about 103, etc.; about 151, about 152, about 153, etc.; about 1,001, about 1,002, etc.; about 10,001, about 10,002, etc. Non-limiting examples of intermediate ranges include about 3 to about 32, about 150 to about 500, or about 5,000 to about 15,000, etc.

It will also be understood that nucleic acid sequences may include additional residues, such as additional 5' or 3' sequences, or various combinations thereof, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein, polypeptide or peptide activity where expression of a proteinaceous composition is
concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' and/or 3' portions of the coding region or may include various internal sequences, i.e., introns, which are known to occur within genes.

Encompassed by the invention are nucleic acid sequences encoding relatively small peptides or fusion peptides, such as, for example, peptides of from about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60, about 61, about 62, about 63, about 64, about 65, about 66, about 67, about 68, about 69, about 70, about 71, about 72, about 73, about 74, about 75, about 76, about 77, about 78, about 79, about 80, about 81, about 82, about 83, about 84, about 85, about 86, about 87, about 88, about 89, about 90, about 91, about 92, about 93, about 94, about 95, about 96, about 97, about 98, about 99, to about 100 amino acids in length, or more preferably, of from about 15 to about 30 amino acids in length; as set forth in SEQ ID NO:2.

IV. Systemic Lupus Erythematosus

A. Disease Manifestations

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease (or autoimmune connective tissue disease) that can affect any part of the body. As occurs in other autoimmune diseases, the immune system attacks the body's cells and tissue, resulting in inflammation and tissue damage. It is a Type III hypersensitivity reaction in which antibody-immune complexes precipitate and cause a further immune response. SLE most often harms the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. The course of the disease is unpredictable, with periods of illness (called "flares") alternating with remissions. A flare has been defined as a measurable increase in disease activity in one or more organ systems involving new or worse clinical signs and symptoms and/or laboratory measurements. It must be considered clinically significant by the assessor and usually there would be at least consideration of a change or an increase in treatment (Ruperto et al., 2010). The disease occurs nine times more often in women than in men, especially in women in
child-bearing years ages 15 to 35, and is also more common in those of non-European
descent.

There is no cure for SLE, and it can be fatal. The leading cause of death is from
cardiovascular diseases acquired from corticosteroid therapy. Survival for people with SLE in
the United States, Canada, and Europe has risen to approximately 95% at five years, 90% at
ten years, and 78% at 20 years, and now approaches that of matched controls without lupus.
Childhood systemic lupus erythematosus generally presents between the ages of 3 and 15,
with girls outnumbering boys 4:1, and typical skin manifestations being butterfly eruption on
the face and photosensitivity.

SLE is one of several diseases known as "the great imitators" because it often mimics
or is mistaken for other illnesses. SLE is a classical item in differential diagnosis, because
SLE symptoms vary widely and come and go unpredictably. Diagnosis can thus be elusive,
with some people suffering unexplained symptoms of untreated SLE for years. Common
initial and chronic complaints include fever, malaise, joint pains, myalgias, fatigue, and
temporary loss of cognitive abilities. Because they are so often seen with other diseases, these
signs and symptoms are not part of the diagnostic criteria for SLE. When occurring in
conjunction with other signs and symptoms (see below), however, they are considered
suggestive.

As many as 30% of sufferers have some dermatological symptoms (65% suffer such
symptoms at some point), with 30% to 50% suffering from the classic malar rash (or butterfly
rash) associated with the disease. Some may exhibit thick, red scaly patches on the skin
(referred to as discoid lupus). Alopecia; mouth, nasal, urinary tract and vaginal ulcers, and
lesions on the skin are also possible manifestations. Tiny tears in delicate tissue around the
eyes can occur after even minimal rubbing.

The most commonly sought medical attention is for joint pain, with the small joints of
the hand and wrist usually affected, although all joints are at risk. The Lupus Foundation
of America estimates more than 90% of those affected will experience joint and/or muscle pain
at some time during the course of their illness. Unlike rheumatoid arthritis, lupus arthritis is
less disabling and usually does not cause severe destruction of the joints. Fewer than 10% of
people with lupus arthritis will develop deformities of the hands and feet. SLE patients are at
particular risk of developing osteoarticular tuberculosis. A possible association between
rheumatoid arthritis and SLE has been suggested, and SLE may be associated with an
increased risk of bone fractures in relatively young women.
Anemia may develop in up to 50% of cases. Low platelet and white blood cell counts may be due to the disease or a side effect of pharmacological treatment. People with SLE may have an association with antiphospholipid antibody syndrome (a thrombotic disorder), wherein autoantibodies to phospholipids are present in their serum. Abnormalities associated with antiphospholipid antibody syndrome include a paradoxical prolonged partial thromboplastin time (which usually occurs in hemorrhagic disorders) and a positive test for antiphospholipid antibodies; the combination of such findings has earned the term "lupus anticoagulant-positive." Another autoantibody finding in SLE is the anticardiolipin antibody, which can cause a false positive test for syphilis.

A person with SLE may have inflammation of various parts of the heart, such as pericarditis, myocarditis, and endocarditis. The endocarditis of SLE is characteristically noninfective (Libman-Sacks endocarditis), and involves either the mitral valve or the tricuspid valve. Atherosclerosis also tends to occur more often and advances more rapidly than in the general population. Lung and pleura inflammation can cause pleuritis, pleural effusion, lupus pneumonitis, chronic diffuse interstitial lung disease, pulmonary hypertension, pulmonary emboli, pulmonary hemorrhage, and shrinking lung syndrome.

Painless hematuria or proteinuria may often be the only presenting renal symptom. Acute or chronic renal impairment may develop with lupus nephritis, leading to acute or end-stage renal failure. Because of early recognition and management of SLE, end-stage renal failure occurs in less than 5% of cases. A histological hallmark of SLE is membranous glomerulonephritis with "wire loop" abnormalities. This finding is due to immune complex deposition along the glomerular basement membrane, leading to a typical granular appearance in immunofluorescence testing.

Neuropsychiatric syndromes can result when SLE affects the central or peripheral nervous systems. The American College of Rheumatology defines 19 neuropsychiatric syndromes in systemic lupus erythematosus. The diagnosis of neuropsychiatric syndromes concurrent with SLE is one of the most difficult challenges in medicine, because it can involve so many different patterns of symptoms, some of which may be mistaken for signs of infectious disease or stroke. The most common neuropsychiatric disorder people with SLE have is headache, although the existence of a specific lupus headache and the optimal approach to headache in SLE cases remains controversial. Other common neuropsychiatric manifestations of SLE include cognitive dysfunction, mood disorder, cerebrovascular disease, seizures, polyneuropathy, anxiety disorder, and psychosis. It can rarely present with intracranial hypertension syndrome, characterized by an elevated intracranial pressure,
papilledema, and headache with occasional abducens nerve paresis, absence of a space-occupying lesion or ventricular enlargement, and normal cerebrospinal fluid chemical and hematological constituents. More rare manifestations are acute confusional state, Guillain-Barre syndrome, aseptic meningitis, autonomic disorder, demyelinating syndrome, mononeuropathy (which might manifest as mononeuritis multiplex), movement disorder (more specifically, chorea), myasthenia gravis, myelopathy, cranial neuropathy and plexopathy. Neural symptoms contribute to a significant percentage of morbidity and mortality in patients with lupus. As a result, the neural side of lupus is being studied in hopes of reducing morbidity and mortality rates. The neural manifestation of lupus is known as neuropsychiatric systemic lupus erythematosus (NPSLE). One aspect of this disease is severe damage to the epithelial cells of the blood-brain barrier.

Lupus has a wide range of symptoms which span the body. The neurological symptoms include headaches, depression, seizures, cognitive dysfunction, mood disorder, cerebrovascular disease, polyneuropathy, anxiety disorder, psychosis, and in some extreme cases, personality disorders.

In certain regions, depression reportedly affects up to 60% of women suffering from SLE. SLE causes an increased rate of fetal death in utero and spontaneous abortion (miscarriage). The overall live-birth rate in SLE patients has been estimated to be 72%. Pregnancy outcome appears to be worse in SLE patients whose disease flares up during pregnancy. Neonatal lupus is the occurrence of SLE symptoms in an infant born from a mother with SLE, most commonly presenting with a rash resembling discoid lupus erythematosus, and sometimes with systemic abnormalities such as heart block or hepatosplenomegaly. Neonatal lupus is usually benign and self-limited.

Fatigue in SLE is probably multifactorial and has been related to not only disease activity or complications such as anemia or hypothyroidism, but also to pain, depression, poor sleep quality, poor physical fitness and lack of social support.

Determining if a patient is in flare can be achieved by applying the Systemic Lupus Erythematosus Disease Activity Index SELENA Modification (world-wide-web at rheumatology.org/Practice/Clinical/Indexes/Systemic_Lupus_Erythematosus_Disease_Activity_Index_SELENA_Modification/). This scale uses a point system to calculate when the accumulated significance of recent changes in various indicators translates into a mild/moderate (SLEDA Index of 3-11 point change) or a severe (12 or more point change) flare.
B. Diagnosis

Antinuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA) form the mainstay of serologic testing for SLE. Several techniques are used to detect ANAs. Clinically the most widely used method is indirect immunofluorescence. The pattern of fluorescence suggests the type of antibody present in the patient's serum. Direct immunofluorescence can detect deposits of immunoglobulins and complement proteins in the patient's skin. When skin not exposed to the sun is tested, a positive direct IF (the so-called Lupus band test) is an evidence of systemic lupus erythematosus.

ANA screening yields positive results in many connective tissue disorders and other autoimmune diseases, and may occur in normal individuals. Subtypes of antinuclear antibodies include anti-Smith and anti-double stranded DNA (dsDNA) antibodies (which are linked to SLE) and anti-histone antibodies (which are linked to drug-induced lupus). Anti-dsDNA antibodies are highly specific for SLE; they are present in 70% of cases, whereas they appear in only 0.5% of people without SLE. The anti-dsDNA antibody titers also tend to reflect disease activity, although not in all cases. Other ANA that may occur in SLE sufferers are anti-U1 RNP (which also appears in systemic sclerosis), SS-A (or anti-Ro) and SS-B (or anti-La; both of which are more common in Sjögren's syndrome). SS-A and SS-B confer a specific risk for heart conduction block in neonatal lupus.

Other tests routinely performed in suspected SLE are complement system levels (low levels suggest consumption by the immune system), electrolytes and renal function (disturbed if the kidney is involved), liver enzymes, and complete blood count. The lupus erythematosus (LE) cell test was commonly used for diagnosis, but it is no longer used because the LE cells are only found in 50-75% of SLE cases, and they are also found in some people with rheumatoid arthritis, scleroderma, and drug sensitivities. Because of this, the LE cell test is now performed only rarely and is mostly of historical significance.

C. Treatment

The treatment of SLE involves preventing flares and reducing their severity and duration when they occur. Treatment can include corticosteroids and anti-malarial drugs. Certain types of lupus nephritis such as diffuse proliferative glomerulonephritis require bouts of cytotoxic drugs. These drugs include cyclophosphamide and mycophenolate. Hydroxychloroquine (HCQ) was approved by the FDA for lupus in 1955. Some drugs approved for other diseases are used for SLE Off-label.' In November 2010, an FDA advisory panel recommended approving belimumab (Benlysta) as a treatment for the pain and
flare-ups common in lupus. The drug was approved by the FDA in March 2011. N-acetylcysteine has been shown to reverse depletion of the natural anti-oxidant glutathione and to safely improve disease activity in a double-blind placebo-controlled pilot study.

Due to the variety of symptoms and organ system involvement with SLE, its severity in an individual must be assessed in order to successfully treat SLE. Mild or remittent disease may, sometimes, be safely left untreated. If required, nonsteroidal anti-inflammatory drugs and antimalarials may be used. Medications such as Prednisone, Cellcept and Prograf have been used in the past. A number of potential treatments are in clinical trials.

Disease-modifying antirheumatic drugs (DMARDs) are used preventively to reduce the incidence of flares, the process of the disease, and lower the need for steroid use; when flares occur, they are treated with corticosteroids. DMARDs commonly in use are antimalarials such as Plaquenil and immunosuppressants (e.g., methotrexate and azathioprine). Plaquenil (hydroxychloroquine) is an FDA-approved antimalarial used for constitutional, cutaneous, and articular manifestations. Hydroxychloroquine has relatively few side effects, and there is evidence that it improves survival among people who have SLE. Cyclophosphamide is used for severe glomerulonephritis or other organ-damaging complications. Mycophenolic acid is also used for treatment of lupus nephritis, but it is not FDA-approved for this indication, and FDA is investigating reports that it may be associated with birth defects when used by pregnant women.

In more severe cases, medications that modulate the immune system (primarily corticosteroids and immunosuppressants) are used to control the disease and prevent recurrence of symptoms (known as flares). Depending on the dosage, people who require steroids may develop Cushing's syndrome, symptoms of which may include obesity, puffy round face, diabetes mellitus, increased appetite, difficulty sleeping and osteoporosis. These may subside if and when the large initial dosage is reduced, but long-term use of even low doses can cause elevated blood pressure and cataracts.

Numerous new immunosuppressive drugs are being actively tested for SLE. Rather than suppressing the immune system nonspecifically, as corticosteroids do, they target the responses of individual [types of] immune cells. Some of these drugs are already FDA-approved for treatment of rheumatoid arthritis. Lupuzor has given encouraging results in a phase lib trial.

Since a large percentage of people with SLE suffer from varying amounts of chronic pain, stronger prescription analgesics (pain killers) may be used if over-the-counter drugs (mainly nonsteroidal anti-inflammatory drugs) do not provide effective relief. Potent NSAIDs
such as indomethacin and diclofenac are relatively contraindicated for patients with SLE because they increase the risk of kidney failure and heart failure.

Moderate pain is typically treated with mild prescription opiates such as dextropropoxyphene and co-codamol. Moderate to severe chronic pain is treated with stronger opioids, such as hydrocodone or longer-acting continuous-release opioids, such as oxycodone, MS Contin, or methadone. The fentanyl duragesic transdermal patch is also a widely used treatment option for the chronic pain caused by complications because of its long-acting timed release and ease of use. When opioids are used for prolonged periods, drug tolerance, chemical dependency, and addiction may occur. Opiate addiction is not typically a concern, since the condition is not likely to ever completely disappear. Thus, lifelong treatment with opioids is fairly common for chronic pain symptoms, accompanied by periodic titration that is typical of any long-term opioid regimen.

Intravenous immunoglobulins may be used to control SLE with organ involvement, or vasculitis. It is believed that they reduce antibody production or promote the clearance of immune complexes from the body, even though their mechanism of action is not well-understood. Unlike immunosuppressives and corticosteroids, IVIGs do not suppress the immune system, so there is less risk of serious infections with these drugs.

Avoiding sunlight is the primary change to the lifestyle of SLE sufferers, as sunlight is known to exacerbate the disease, as is the debilitating effect of intense fatigue. These two problems can lead to patients becoming housebound for long periods of time. Drugs unrelated to SLE should be prescribed only when known not to exacerbate the disease. Occupational exposure to silica, pesticides and mercury can also make the disease worsen.

Renal transplants are the treatment of choice for end-stage renal disease, which is one of the complications of lupus nephritis, but the recurrence of the full disease is common in up to 30% of patients.

Antiphospholipid syndrome is also related to the onset of neural lupus symptoms in the brain. In this form of the disease the cause is very different from lupus: thromboses (blood clots or "sticky blood") form in blood vessels, which prove to be fatal if they move within the blood stream. If the thromboses migrate to the brain, they can potentially cause a stroke by blocking the blood supply to the brain.

If this disorder is suspected in patients, brain scans are usually required for early detection. These scans can show localized areas of the brain where blood supply has not been adequate. The treatment plan for these patients requires anticoagulation. Often, low-dose
aspirin is prescribed for this purpose, although for cases involving thrombosis anticoagulants such as warfarin are used.

V. Assessing ARID3a Expression

Thus, in accordance with the present invention, methods are provided for the assaying of expression of ARID3a. As discussed above, the principle applications are to (a) determine if a patient has SLE as opposed to a distinct autoimmune condition, (b) to determine the severity of the disease, (c) to determine the current intensity of the inflammatory state, and (d) to predict or assess the efficacy of a. In each of these assays, the expression of ARID3a will be measured, and in some, the expression is measured multiple times to assess not only absolute values, but changes in these values overtime. Virtually any method of measuring gene expression may be utilized, and the following discussion is exemplary in nature and in no way limiting.

A. Immunologic Assays

There are a variety of methods that can be used to assess protein expression. One such approach is to perform protein identification with the use of antibodies. As used herein, the term "antibody" is intended to refer broadly to any immunologic binding agent such as IgG, IgM, IgA, IgD and IgE. Generally, IgG and/or IgM are preferred because they are the most common antibodies in the physiological situation and because they are most easily made in a laboratory setting. The term "antibody" also refers to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')2, single domain antibodies (DABs), Fv, scFv (single chain Fv), and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Means for preparing and characterizing antibodies, both polyclonal and monoclonal, are also well known in the art (See, e.g., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; incorporated herein by reference). In particular, antibodies to calcyclin, calpactin I light chain, astrocytic phosphoprotein PEA-15 and tubulin-specific chaperone A are contemplated.

In accordance with the present invention, immunodetection methods are provided. Some immunodetection methods include enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoradiometric assay, fluoroimmunoassay, chemiluminescent assay, bioluminescent assay, and Western blot to mention a few. The steps of various useful immunodetection methods have been described in the scientific literature, such as, e.g.,
Doolittle & Ben-Zeev O, 1999; Gulbis & Galand, 1993; De Jager et al, 1993; and Nakamura et al., 1987, each incorporated herein by reference.

In general, the immunobinding methods include obtaining a sample suspected of containing a relevant polypeptide, and contacting the sample with a first antibody under conditions effective to allow the formation of immunocomplexes. In terms of antigen detection, the biological sample analyzed may be any sample that is suspected of containing an antigen, such as, for example, a tissue section or specimen, a homogenized tissue extract, a cell, or even a biological fluid.

Contacting the chosen biological sample with the antibody under effective conditions and for a period of time sufficient to allow the formation of immune complexes (primary immune complexes) is generally a matter of simply adding the antibody composition to the sample and incubating the mixture for a period of time long enough for the antibodies to form immune complexes with, i.e., to bind to, any antigens present. After this time, the sample-antibody composition, such as a tissue section, ELISA plate, dot blot or western blot, will generally be washed to remove any non-specifically bound antibody species, allowing only those antibodies specifically bound within the primary immune complexes to be detected.

In general, the detection of immunocomplex formation is well known in the art and may be achieved through the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any of those radioactive, fluorescent, biological and enzymatic tags. Patents concerning the use of such labels include U.S. Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241, each incorporated herein by reference. Of course, one may find additional advantages through the use of a secondary binding ligand such as a second antibody and/or a biotin/avidin ligand binding arrangement, as is known in the art.

The antibody employed in the detection may itself be linked to a detectable label, wherein one would then simply detect this label, thereby allowing the amount of the primary immune complexes in the composition to be determined. Alternatively, the first antibody that becomes bound within the primary immune complexes may be detected by means of a second binding ligand that has binding affinity for the antibody. In these cases, the second binding ligand may be linked to a detectable label. The second binding ligand is itself often an antibody, which may thus be termed a "secondary" antibody. The primary immune complexes are contacted with the labeled, secondary binding ligand, or antibody, under effective conditions and for a period of time sufficient to allow the formation of secondary immune complexes. The secondary immune complexes are then generally washed to remove
any non-specifically bound labeled secondary antibodies or ligands, and the remaining label in the secondary immune complexes is then detected.

Further methods include the detection of primary immune complexes by a two step approach. A second binding ligand, such as an antibody, that has binding affinity for the antibody is used to form secondary immune complexes, as described above. After washing, the secondary immune complexes are contacted with a third binding ligand or antibody that has binding affinity for the second antibody, again under effective conditions and for a period of time sufficient to allow the formation of immune complexes (tertiary immune complexes). The third ligand or antibody is linked to a detectable label, allowing detection of the tertiary immune complexes thus formed. This system may provide for signal amplification if this is desired.

One method of immunodetection designed by Charles Cantor uses two different antibodies. A first step biotinylated, monoclonal or polyclonal antibody is used to detect the target antigen(s), and a second step antibody is then used to detect the biotin attached to the complexed biotin. In that method the sample to be tested is first incubated in a solution containing the first step antibody. If the target antigen is present, some of the antibody binds to the antigen to form a biotinylated antibody/antigen complex. The antibody/antigen complex is then amplified by incubation in successive solutions of streptavidin (or avidin), biotinylated DNA, and/or complementary biotinylated DNA, with each step adding additional biotin sites to the antibody/antigen complex. The amplification steps are repeated until a suitable level of amplification is achieved, at which point the sample is incubated in a solution containing the second step antibody against biotin. This second step antibody is labeled, as for example with an enzyme that can be used to detect the presence of the antibody/antigen complex by histoenzymology using a chromogen substrate. With suitable amplification, a conjugate can be produced which is macroscopically visible.

Another known method of immunodetection takes advantage of the immuno-PCR (Polymerase Chain Reaction) methodology. The PCR method is similar to the Cantor method up to the incubation with biotinylated DNA, however, instead of using multiple rounds of streptavidin and biotinylated DNA incubation, the DNA/biotin/streptavidin/antibody complex is washed out with a low pH or high salt buffer that releases the antibody. The resulting wash solution is then used to carry out a PCR reaction with suitable primers with appropriate controls. At least in theory, the enormous amplification capability and specificity of PCR can be utilized to detect a single antigen molecule.
As detailed above, immunoassays are in essence binding assays. Certain immunoassays are the various types of enzyme linked immunosorbent assays (ELISAs) and radioimmunoassays (RIA) known in the art. However, it will be readily appreciated that detection is not limited to such techniques, and Western blotting, dot blotting, FACS analyses, and the like may also be used.

In one exemplary ELISA, the antibodies of the invention are immobilized onto a selected surface exhibiting protein affinity, such as a well in a polystyrene microtiter plate. Then, a test composition suspected of containing the antigen, such as a clinical sample, is added to the wells. After binding and washing to remove non-specifically bound immune complexes, the bound antigen may be detected. Detection is generally achieved by the addition of another antibody that is linked to a detectable label. This type of ELISA is a simple "sandwich ELISA." Detection may also be achieved by the addition of a second antibody, followed by the addition of a third antibody that has binding affinity for the second antibody, with the third antibody being linked to a detectable label.

In another exemplary ELISA, the samples suspected of containing the antigen are immobilized onto the well surface and then contacted with the anti-ORF message and anti-ORF translated product antibodies of the invention. After binding and washing to remove non-specifically bound immune complexes, the bound anti-ORF message and anti-ORF translated product antibodies are detected. Where the initial anti-ORF message and anti-ORF translated product antibodies are linked to a detectable label, the immune complexes may be detected directly. Again, the immune complexes may be detected using a second antibody that has binding affinity for the first anti-ORF message and anti-ORF translated product antibody, with the second antibody being linked to a detectable label.

Another ELISA in which the antigens are immobilized, involves the use of antibody competition in the detection. In this ELISA, labeled antibodies against an antigen are added to the wells, allowed to bind, and detected by means of their label. The amount of an antigen in an unknown sample is then determined by mixing the sample with the labeled antibodies against the antigen during incubation with coated wells. The presence of an antigen in the sample acts to reduce the amount of antibody against the antigen available for binding to the well and thus reduces the ultimate signal. This is also appropriate for detecting antibodies against an antigen in an unknown sample, where the unlabeled antibodies bind to the antigen-coated wells and also reduces the amount of antigen available to bind the labeled antibodies.

"Under conditions effective to allow immune complex (antigen/antibody) formation" means that the conditions preferably include diluting the antigens and/or antibodies with
solutions such as BSA, bovine gamma globulin (BGG) or phosphate buffered saline (PBS)/Tween. These added agents also tend to assist in the reduction of nonspecific background. The "suitable" conditions also mean that the incubation is at a temperature or for a period of time sufficient to allow effective binding. Incubation steps are typically from about 1 to 2 to 4 hours or so, at temperatures preferably on the order of 25°C to 27°C, or may be overnight at about 4°C or so.

Another antibody-based approach to assessing ARID3a expression is Fluorescence-Activated Cell Sorting (FACS), a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. It provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest. A cell suspension is entrained in the center of a narrow, rapidly flowing stream of liquid. The flow is arranged so that there is a large separation between cells relative to their diameter. A vibrating mechanism causes the stream of cells to break into individual droplets. The system is adjusted so that there is a low probability of more than one cell per droplet. Just before the stream breaks into droplets, the flow passes through a fluorescence measuring station where the fluorescent character of interest of each cell is measured. An electrical charging ring is placed just at the point where the stream breaks into droplets. A charge is placed on the ring based on the immediately prior fluorescence intensity measurement, and the opposite charge is trapped on the droplet as it breaks from the stream. The charged droplets then fall through an electrostatic deflection system that diverts droplets into containers based upon their charge. In some systems, the charge is applied directly to the stream, and the droplet breaking off retains charge of the same sign as the stream. The stream is then returned to neutral after the droplet breaks off. One common way to use FAC is with a fluorescently labeled antibody that binds to a target on or in a cell, thereby identifying cells with a given target. This technique can be used quantitatively where the amount of fluorescent activity correlates to the amount of target, thereby permitting one to sort based on relative amounts of fluorescence, and hence relative amounts of the target.

B. Mass Spectrometry

By exploiting the intrinsic properties of mass and charge, mass spectrometry (MS) can resolved and confidently identified a wide variety of complex compounds, including proteins. Traditional quantitative MS has used electrospray ionization (ESI) followed by
tandem MS (MS/MS) while newer quantitative methods are being developed using matrix assisted laser desorption/ionization (MALDI) followed by time of flight (TOF) MS. In accordance with the present invention, one can generate mass spectrometry profiles that are useful for grading gliomas and predicting glioma patient survival, without regard for the identity of specific proteins. Alternatively, given the established links with calcyclin, calpain I light chain, astrocytic phosphoprotein PEA-15 and tubulin-specific chaperone A, mass spectrometry may be used to look for the levels of these proteins particularly.

ESI is a convenient ionization technique that is used to produce gaseous ions from highly polar, mostly nonvolatile biomolecules, including lipids. The sample is injected as a liquid at low flow rates (1-10 μL/min) through a capillary tube to which a strong electric field is applied. The field generates additional charges to the liquid at the end of the capillary and produces a fine spray of highly charged droplets that are electrostatically attracted to the mass spectrometer inlet. The evaporation of the solvent from the surface of a droplet as it travels through the desolvation chamber increases its charge density substantially. When this increase exceeds the Rayleigh stability limit, ions are ejected and ready for MS analysis.

In ESI tandem mass spectroscopy (ESI/MS/MS), one is able to simultaneously analyze both precursor ions and product ions, thereby monitoring a single precursor product reaction and producing (through selective reaction monitoring (SRM)) a signal only when the desired precursor ion is present. When the internal standard is a stable isotope-labeled version of the analyte, this is known as quantification by the stable isotope dilution method. This approach has been used to accurately measure pharmaceuticals and bioactive peptides. Newer methods are performed on widely available MALDI-TOF instruments, which can resolve a wider mass range and have been used to quantify metabolites, peptides, and proteins. Larger molecules such as peptides can be quantified using unlabeled homologous peptides as long as their chemistry is similar to the analyte peptide. Protein quantification has been achieved by quantifying tryptic peptides. Complex mixtures such as crude extracts can be analyzed, but in some instances sample clean up is required.

Secondary ion mass spectroscopy, or SIMS, is an analytical method that uses ionized particles emitted from a surface for mass spectroscopy at a sensitivity of detection of a few parts per billion. The sample surface is bombarded by primary energetic particles, such as electrons, ions (e.g., O, Cs), neutrals or even photons, forcing atomic and molecular particles to be ejected from the surface, a process called sputtering. Since some of these sputtered particles carry a charge, a mass spectrometer can be used to measure their mass and charge. Continued sputtering permits measuring of the exposed elements as material is removed.
This in turn permits one to construct elemental depth profiles. Although the majority of secondary ionized particles are electrons, it is the secondary ions which are detected and analysis by the mass spectrometer in this method.

Laser desorption mass spectroscopy (LD-MS) involves the use of a pulsed laser, which induces desorption of sample material from a sample site - effectively, this means vaporization of sample off of the sample substrate. This method is usually only used in conjunction with a mass spectrometer, and can be performed simultaneously with ionization if one uses the right laser radiation wavelength.

When coupled with Time-of-Flight (TOF) measurement, LD-MS is referred to as LDLPMS (Laser Desorption Laser Photoionization Mass Spectroscopy). The LDLPMS method of analysis gives instantaneous volatilization of the sample, and this form of sample fragmentation permits rapid analysis without any wet extraction chemistry. The LDLPMS instrumentation provides a profile of the species present while the retention time is low and the sample size is small. In LDLPMS, an impactor strip is loaded into a vacuum chamber. The pulsed laser is fired upon a certain spot of the sample site, and species present are desorbed and ionized by the laser radiation. This ionization also causes the molecules to break up into smaller fragment-ions. The positive or negative ions made are then accelerated into the flight tube, being detected at the end by a microchannel plate detector. Signal intensity, or peak height, is measured as a function of travel time. The applied voltage and charge of the particular ion determines the kinetic energy, and separation of fragments is due to different size causing different velocity. Each ion mass will thus have a different flight-time to the detector.

One can either form positive ions or negative ions for analysis. Positive ions are made from regular direct photoionization, but negative ion formation requires a higher powered laser and a secondary process to gain electrons. Most of the molecules that come off the sample site are neutrals, and thus can attract electrons based on their electron affinity. The negative ion formation process is less efficient than forming just positive ions. The sample constituents will also affect the outlook of a negative ion spectrum.

Other advantages with the LDLPMS method include the possibility of constructing the system to give a quiet baseline of the spectra because one can prevent coevolved neutrals from entering the flight tube by operating the instrument in a linear mode. Also, in environmental analysis, the salts in the air and as deposits will not interfere with the laser desorption and ionization. This instrumentation also is very sensitive, known to detect trace levels in natural samples without any prior extraction preparations.
Since its inception and commercial availability, the versatility of MALDI-TOF-MS has been demonstrated convincingly by its extensive use for qualitative analysis. For example, MALDI-TOF-MS has been employed for the characterization of synthetic polymers, peptide and protein analysis, DNA oligonucleotide sequencing, and the characterization of recombinant proteins. Recently, applications of MALDI-TOF-MS have been extended to include the direct analysis of biological tissues and single cell organisms with the aim of characterizing endogenous peptide and protein constituents.

The properties that make MALDI-TOF-MS a popular qualitative tool—its ability to analyze molecules across an extensive mass range, high sensitivity, minimal sample preparation and rapid analysis times—also make it a potentially useful quantitative tool. MALDI-TOF-MS also enables non-volatile and thermally labile molecules to be analyzed with relative ease. It is therefore prudent to explore the potential of MALDI-TOF-MS for quantitative analysis in clinical settings, for toxicological screenings, as well as for environmental analysis. In addition, the application of MALDI-TOF-MS to the quantification of peptides and proteins is particularly relevant. The ability to quantify intact proteins in biological tissue and fluids presents a particular challenge in the expanding area of proteomics and investigators urgently require methods to accurately measure the absolute quantity of proteins. While there have been reports of quantitative MALDI-TOF-MS applications, there are many problems inherent to the MALDI ionization process that have restricted its widespread use. These limitations primarily stem from factors such as the sample/matrix heterogeneity, which are believed to contribute to the large variability in observed signal intensities for analytes, the limited dynamic range due to detector saturation, and difficulties associated with coupling MALDI-TOF-MS to on-line separation techniques such as liquid chromatography. Combined, these factors are thought to compromise the accuracy, precision, and utility with which quantitative determinations can be made.

Because of these difficulties, practical examples of quantitative applications of MALDI-TOF-MS have been limited. Most of the studies to date have focused on the quantification of low mass analytes, in particular, alkaloids or active ingredients in agricultural or food products, whereas other studies have demonstrated the potential of MALDI-TOF-MS for the quantification of biologically relevant analytes such as neuropeptides, proteins, antibiotics, or various metabolites in biological tissue or fluid. In earlier work it was shown that linear calibration curves could be generated by MALDI-TOF-MS provided that an appropriate internal standard was employed. This standard can “correct"
for both sample-to-sample and shot-to-shot variability. Stable isotope labeled internal standards (isotopomers) give the best result.

With the marked improvement in resolution available on modern commercial instruments, primarily because of delayed extraction, the opportunity to extend quantitative work to other examples is now possible; not only of low mass analytes, but also biopolymers. Of particular interest is the prospect of absolute multi-component quantification in biological samples (e.g., proteomics applications).

The properties of the matrix material used in the MALDI method are critical. Only a select group of compounds is useful for the selective desorption of proteins and polypeptides. A review of all the matrix materials available for peptides and proteins shows that there are certain characteristics the compounds must share to be analytically useful. Despite its importance, very little is known about what makes a matrix material "successful" for MALDI. The few materials that do work well are used heavily by all MALDI practitioners and new molecules are constantly being evaluated as potential matrix candidates. With a few exceptions, most of the matrix materials used are solid organic acids. Liquid matrices have also been investigated, but are not used routinely.

C. Nucleic Acid Detection

In alternative embodiments for detecting protein expression, one may assay for gene transcription. For example, an indirect method for detecting protein expression is to detect mRNA transcripts from which the proteins are made. The following is a discussion of such methods, which are applicable particularly to calcyclin, calpactin I light chain, astrocytic phosphoprotein PEA-15 and tubulin-specific chaperone A in the context of the present invention.

1. Hybridization

There are a variety of ways by which one can assess gene expression. These methods either look at protein or at mRNA levels. Methods looking at mRNAs all fundamentally rely, at a basic level, on nucleic acid hybridization. Hybridization is defined as the ability of a nucleic acid to selectively form duplex molecules with complementary stretches of DNAs and/or RNAs. Depending on the application envisioned, one would employ varying conditions of hybridization to achieve varying degrees of selectivity of the probe or primers for the target sequence.

Typically, a probe or primer of between 13 and 100 nucleotides, preferably between 17 and 100 nucleotides in length up to 1-2 kilobases or more in length will allow the formation of a duplex molecule that is both stable and selective. Molecules having complementary sequences
over contiguous stretches greater than 20 bases in length are generally preferred, to increase stability and selectivity of the hybrid molecules obtained. One will generally prefer to design nucleic acid molecules for hybridization having one or more complementary sequences of 20 to 30 nucleotides, or even longer where desired. Such fragments may be readily prepared, for example, by directly synthesizing the fragment by chemical means or by introducing selected sequences into recombinant vectors for recombinant production.

For applications requiring high selectivity, one will typically desire to employ relatively high stringency conditions to form the hybrids. For example, relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.10 M NaCl at temperatures of about 50°C to about 70°C. Such high stringency conditions tolerate little, if any, mismatch between the probe or primers and the template or target strand and would be particularly suitable for isolating specific genes or for detecting specific mRNA transcripts. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

For certain applications, for example, lower stringency conditions may be used. Under these conditions, hybridization may occur even though the sequences of the hybridizing strands are not perfectly complementary, but are mismatched at one or more positions. Conditions may be rendered less stringent by increasing salt concentration and/or decreasing temperature. For example, a medium stringency condition could be provided by about 0.1 to 0.25 M NaCl at temperatures of about 37°C to about 55°C, while a low stringency condition could be provided by about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Hybridization conditions can be readily manipulated depending on the desired results.

In other embodiments, hybridization may be achieved under conditions of, for example, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl\(_2\), 1.0 mM dithiothreitol, at temperatures between approximately 20°C to about 37°C. Other hybridization conditions utilized could include approximately 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl\(_2\), at temperatures ranging from approximately 40°C to about 72°C.

In certain embodiments, it will be advantageous to employ nucleic acids of defined sequences of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of being detected. In preferred embodiments, one may desire to employ a fluorescent label or an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmentally undesirable reagents. In the case of enzyme tags,
colorimetric indicator substrates are known that can be employed to provide a detection means that is visibly or spectrophotometrically detectable, to identify specific hybridization with complementary nucleic acid containing samples.

In general, it is envisioned that the probes or primers described herein will be useful as reagents in solution hybridization, as in PCR™, for detection of expression of corresponding genes, as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to hybridization with selected probes under desired conditions. The conditions selected will depend on the particular circumstances (depending, for example, on the G+C content, type of target nucleic acid, source of nucleic acid, size of hybridization probe, etc.). Optimization of hybridization conditions for the particular application of interest is well known to those of skill in the art. After washing of the hybridized molecules to remove non-specifically bound probe molecules, hybridization is detected, and/or quantified, by determining the amount of bound label. Representative solid phase hybridization methods are disclosed in U.S. Patents 5,843,663, 5,900,481 and 5,919,626. Other methods of hybridization that may be used in the practice of the present invention are disclosed in U.S. Patents 5,849,481, 5,849,486 and 5,851,772. The relevant portions of these and other references identified in this section of the Specification are incorporated herein by reference.

2. Amplification of Nucleic Acids

Since many mRNAs are present in relatively low abundance, nucleic acid amplification greatly enhances the ability to assess expression. The general concept is that nucleic acids can be amplified using paired primers flanking the region of interest. The term "primer," as used herein, is meant to encompass any nucleic acid that is capable of priming the synthesis of a nascent nucleic acid in a template-dependent process. Typically, primers are oligonucleotides from ten to twenty and/or thirty base pairs in length, but longer sequences can be employed. Primers may be provided in double-stranded and/or single-stranded form, although the single-stranded form is preferred.

Pairs of primers designed to selectively hybridize to nucleic acids corresponding to selected genes are contacted with the template nucleic acid under conditions that permit selective hybridization. Depending upon the desired application, high stringency hybridization conditions may be selected that will only allow hybridization to sequences that
are completely complementary to the primers. In other embodiments, hybridization may occur under reduced stringency to allow for amplification of nucleic acids contain one or more mismatches with the primer sequences. Once hybridized, the template-primer complex is contacted with one or more enzymes that facilitate template-dependent nucleic acid synthesis. Multiple rounds of amplification, also referred to as "cycles," are conducted until a sufficient amount of amplification product is produced.

The amplification product may be detected or quantified. In certain applications, the detection may be performed by visual means. Alternatively, the detection may involve indirect identification of the product via chemilluminescence, radioactive scintigraphy of incorporated radiolabel or fluorescent label or even via a system using electrical and/or thermal impulse signals.

A number of template dependent processes are available to amplify the oligonucleotide sequences present in a given template sample. One of the best known amplification methods is the polymerase chain reaction (referred to as PCR™) which is described in detail in U.S. Patents 4,683,195, 4,683,202 and 4,800,159, and in Innis et al, 1988, each of which is incorporated herein by reference in their entirety.

A reverse transcriptase PCR™ amplification procedure may be performed to quantify the amount of mRNA amplified. Methods of reverse transcribing RNA into cDNA are well known (see Sambrook et al, 1989). Alternative methods for reverse transcription utilize thermostable DNA polymerases. These methods are described in WO 90/07641. Polymerase chain reaction methodologies are well known in the art. Representative methods of RT-PCR are described in U.S. Patent 5,882,864.

Whereas standard PCR usually uses one pair of primers to amplify a specific sequence, multiplex-PCR (MPCR) uses multiple pairs of primers to amplify many sequences simultaneously. The presence of many PCR primers in a single tube could cause many problems, such as the increased formation of misprimed PCR products and "primer dimers", the amplification discrimination of longer DNA fragment and so on. Normally, MPCR buffers contain a Taq Polymerase additive, which decreases the competition among amplicons and the amplification discrimination of longer DNA fragment during MPCR. MPCR products can further be hybridized with gene-specific probe for verification. Theoretically, one should be able to use as many as primers as necessary. However, due to side effects (primer dimers, misprimed PCR products, etc.) caused during MPCR, there is a limit (less than 20) to the number of primers that can be used in a MPCR reaction. See also European Application No. 0 364 255 and Mueller and Wold (1989).
Another method for amplification is ligase chain reaction ("LCR"), disclosed in European Application No. 320 308, incorporated herein by reference in its entirety. U.S. Patent 4,883,750 describes a method similar to LCR for binding probe pairs to a target sequence. A method based on PCR™ and oligonucleotide ligase assay (OLA), disclosed in U.S. Patent 5,912,148, may also be used.

Alternative methods for amplification of target nucleic acid sequences that may be used in the practice of the present invention are disclosed in U.S. Patents 5,843,650, 5,846,709, 5,846,783, 5,849,546, 5,849,497, 5,849,547, 5,858,652, 5,866,366, 5,916,776, 5,922,574, 5,928,905, 5,928,906, 5,932,451, 5,935,825, 5,939,291 and 5,942,391, GB Application No. 2 202 328, and in PCT Application No. PCT/US89/01025, each of which is incorporated herein by reference in its entirety.

Qbeta Replicase, described in PCT Application No. PCT/US87/00880, may also be used as an amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence which may then be detected.

An isothermal amplification method, in which restriction endonucleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'-[alpha-thio]-triphosphates in one strand of a restriction site may also be useful in the amplification of nucleic acids in the present invention (Walker et al., 1992). Strand Displacement Amplification (SDA), disclosed in U.S. Patent 5,916,779, is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, i.e., nick translation.

Other nucleic acid amplification procedures include transcription-based amplification systems (TAS), including nucleic acid sequence based amplification (NASBA) and 3SR (Kwoh et al., 1989; Gingeras et al., PCT Application WO 88/10315, incorporated herein by reference in their entirety). European Application No. 329 822 disclose a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention.

PCT Application WO 89/06700 (incorporated herein by reference in its entirety) disclose a nucleic acid sequence amplification scheme based on the hybridization of a promoter region/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic, i.e., new
templates are not produced from the resultant RNA transcripts. Other amplification methods include "race" and "one-sided PCR" (Frohman, 1990; Ohara et al., 1989).

3. Detection of Nucleic Acids

Following any amplification, it may be desirable to separate the amplification product from the template and/or the excess primer. In one embodiment, amplification products are separated by agarose, agarose-acrylamide or polyacrylamide gel electrophoresis using standard methods (Sambrook et al., 1989). Separated amplification products may be cut out and eluted from the gel for further manipulation. Using low melting point agarose gels, the separated band may be removed by heating the gel, followed by extraction of the nucleic acid.

Separation of nucleic acids may also be effected by chromatographic techniques known in art. There are many kinds of chromatography which may be used in the practice of the present invention, including adsorption, partition, ion-exchange, hydroxylapatite, molecular sieve, reverse-phase, column, paper, thin-layer, and gas chromatography as well as HPLC.

In certain embodiments, the amplification products are visualized. A typical visualization method involves staining of a gel with ethidium bromide and visualization of bands under UV light. Alternatively, if the amplification products are integrally labeled with radio- or fluorometrically-labeled nucleotides, the separated amplification products can be exposed to x-ray film or visualized under the appropriate excitatory spectra.

In one embodiment, following separation of amplification products, a labeled nucleic acid probe is brought into contact with the amplified marker sequence. The probe preferably is conjugated to a chromophore but may be radiolabeled. In another embodiment, the probe is conjugated to a binding partner, such as an antibody or biotin, or another binding partner carrying a detectable moiety.

In particular embodiments, detection is by Southern blotting and hybridization with a labeled probe. The techniques involved in Southern blotting are well known to those of skill in the art (see Sambrook et al., 2001). One example of the foregoing is described in U.S. Patent 5,279,721, incorporated by reference herein, which discloses an apparatus and method for the automated electrophoresis and transfer of nucleic acids. The apparatus permits electrophoresis and blotting without external manipulation of the gel and is ideally suited to carrying out methods according to the present invention.
Other methods of nucleic acid detection that may be used in the practice of the instant
invention are disclosed in U.S. Patents 5,840,873, 5,843,640, 5,843,651, 5,846,708,
5,846,717, 5,846,726, 5,846,729, 5,849,487, 5,853,990, 5,853,992, 5,853,993, 5,856,092,
5,861,244, 5,863,732, 5,863,753, 5,866,331, 5,905,024, 5,910,407, 5,912,124, 5,912,145,
5,919,630, 5,925,517, 5,928,862, 5,928,869, 5,929,227, 5,932,413 and 5,935,791, each of
which is incorporated herein by reference.

4. Nucleic Acid Arrays

Microarrays comprise a plurality of polymeric molecules spatially distributed over,
and stably associated with, the surface of a substantially planar substrate, e.g., biochips.
Microarrays of polynucleotides have been developed and find use in a variety of applications,
such as screening and DNA sequencing. One area in particular in which microarrays find use
is in gene expression analysis.

In gene expression analysis with microarrays, an array of "probe" oligonucleotides is
contacted with a nucleic acid sample of interest, i.e., target, such as polyA mRNA from a
particular tissue type. Contact is carried out under hybridization conditions and unbound
nucleic acid is then removed. The resultant pattern of hybridized nucleic acid provides
information regarding the genetic profile of the sample tested. Methodologies of gene
expression analysis on microarrays are capable of providing both qualitative and quantitative
information.

A variety of different arrays which may be used are known in the art. The probe
molecules of the arrays which are capable of sequence specific hybridization with target
nucleic acid may be polynucleotides or hybridizing analogues or mimetics thereof, including:
nucleic acids in which the phosphodiester linkage has been replaced with a substitute linkage,
such as phosphorothioate; methylphosphonate, phosphoramidate, and the like; nucleic acids in which the ribose subunit has been substituted, e.g., hexose
phosphodiester; peptide nucleic acids; and the like. The length of the probes will generally
range from 10 to 1000 nts, where in some embodiments the probes will be oligonucleotides
and usually range from 15 to 150 nts and more usually from 15 to 100 nts in length, and in
other embodiments the probes will be longer, usually ranging in length from 150 to 1000 nts,
where the polynucleotide probes may be single- or double-stranded, usually single-stranded,
and may be PCR fragments amplified from cDNA.

The probe molecules on the surface of the substrates will correspond to selected genes
being analyzed and be positioned on the array at a known location so that positive
hybridization events may be correlated to expression of a particular gene in the physiological
source from which the target nucleic acid sample is derived. The substrates with which the
probe molecules are stably associated may be fabricated from a variety of materials,
including plastics, ceramics, metals, gels, membranes, glasses, and the like. The arrays may
be produced according to any convenient methodology, such as preforming the probes and
then stably associating them with the surface of the support or growing the probes directly on
the support. A number of different array configurations and methods for their production are
known to those of skill in the art and disclosed in U.S. Patents 5,445,934, 5,532,128,
5,556,752, 5,242,974, 5,384,261, 5,405,783, 5,412,087, 5,424,186, 5,429,807, 5,436,327,
5,472,672, 5,527,681, 5,529,756, 5,545,531, 5,554,501, 5,561,071, 5,571,639, 5,593,839,
5,599,695, 5,624,711, 5,658,734, 5,700,637, and 6,004,755.

Following hybridization, where non-hybridized labeled nucleic acid is capable of
emitting a signal during the detection step, a washing step is employed where unhybridized
labeled nucleic acid is removed from the support surface, generating a pattern of hybridized
nucleic acid on the substrate surface. A variety of wash solutions and protocols for their use
are known to those of skill in the art and may be used.

Where the label on the target nucleic acid is not directly detectable, one then contacts
the array, now comprising bound target, with the other member(s) of the signal producing
system that is being employed. For example, where the label on the target is biotin, one then
contacts the array with streptavidin-fluorescer conjugate under conditions sufficient for
binding between the specific binding member pairs to occur. Following contact, any unbound
members of the signal producing system will then be removed, e.g., by washing. The specific
wash conditions employed will necessarily depend on the specific nature of the signal
producing system that is employed, and will be known to those of skill in the art familiar with
the particular signal producing system employed.

The resultant hybridization pattern(s) of labeled nucleic acids may be visualized or
detected in a variety of ways, with the particular manner of detection being chosen based on
the particular label of the nucleic acid, where representative detection means include
scintillation counting, autoradiography, fluorescence measurement, calorimetric
measurement, light emission measurement and the like.

Prior to detection or visualization, where one desires to reduce the potential for a
mismatch hybridization event to generate a false positive signal on the pattern, the array of
hybridized target/probe complexes may be treated with an endonuclease under conditions
sufficient such that the endonuclease degrades single stranded, but not double stranded DNA.
A variety of different endonucleases are known and may be used, where such nucleases include: mung bean nuclease, S1 nuclease, and the like. Where such treatment is employed in an assay in which the target nucleic acids are not labeled with a directly detectable label, e.g., in an assay with biotinylated target nucleic acids, the endonuclease treatment will generally be performed prior to contact of the array with the other member(s) of the signal producing system, e.g., fluorescent-streptavidin conjugate. Endonuclease treatment, as described above, ensures that only end-labeled target/probe complexes having a substantially complete hybridization at the 3’ end of the probe are detected in the hybridization pattern.

Following hybridization and any washing step(s) and/or subsequent treatments, as described above, the resultant hybridization pattern is detected. In detecting or visualizing the hybridization pattern, the intensity or signal value of the label will be not only be detected but quantified, by which is meant that the signal from each spot of the hybridization will be measured and compared to a unit value corresponding the signal emitted by known number of end-labeled target nucleic acids to obtain a count or absolute value of the copy number of each end-labeled target that is hybridized to a particular spot on the array in the hybridization pattern.

VI. Treating SLE

The present invention contemplates the treatment of SLE using either standard therapeutic approaches, and/or ARID3a inhibition. Advantages accruing to the present invention include earlier intervention, when ARID3a levels are rising or have risen, but the symptoms of excessive B cell activity have not appeared. Also, the present disclosure is the first connection between ARID3a and SLE, thus opening a new possible therapeutic target in this challenging disease.

When given in combination, these compositions one would generally be administered in a combined amount effective to achieve a reduction in one or more SLE disease parameter. This process may involve contacting the subject with the both agents/therapies at the same time, e.g., using a single composition or pharmacological formulation that includes both agents, or by contacting the subject with two distinct compositions or formulations, at the same time. Alternatively, one treatment may precede or follow the other treatment by intervals ranging from minutes to weeks. One would generally ensure that a significant period of time did not expire between the time of each delivery, such that the therapies would still be able to exert an advantageously combined effect on the cell/subject. In such instances, it is contemplated that one would contact the cell with both modalities within about
12-24 hours of each other, within about 6-12 hours of each other, or with a delay time of only about 12 hours. In some situations, it may be desirable to extend the time period for treatment significantly; however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

It also is conceivable that more than one administration of either therapy will be desired. Various combinations may be employed, where the standard therapy (corticosteroid or anti-malarial drug) is "A," and the anti-ARID2a therapy is "B,” as exemplified below:

A/B/A B/A/B B/B/A A/A/B B/A/A A/B/B B/B/A A/B/A B/B/A
A/A/B/A B/A/A/B A/B/A/A A/B/A/A A/A/B/B A/B/B/A B/B/A/B

Other combinations are contemplated.

A. Pharmaceutical Compositions and Administration

Where therapeutic applications are contemplated, it will be necessary to prepare pharmaceutical compositions in a form appropriate for the intended application. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

One will generally desire to employ appropriate salts and buffers to render delivery vectors stable and allow for uptake by target cells. Buffers also will be employed when recombinant cells are introduced into a patient. Aqueous compositions of the present invention comprise an effective amount of the vector to cells, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions also are referred to as inocula. The phrase "pharmaceutically or pharmacologically acceptable” refers to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the vectors or cells of the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

The active compositions of the present invention may include classic pharmaceutical preparations. Administration of these compositions according to the present invention will be
via any common route so long as the target tissue is available via that route. Such routes include oral, nasal, buccal, rectal, vaginal or topical route. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, or intravenous injection. Such compositions would normally be administered as pharmaceutically acceptable compositions.

The active compounds may also be administered parenterally or intraperitoneally. Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a
powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

For oral administration the polypeptides of the present invention may be incorporated with excipients and used in the form of non-ingestible mouthwashes and dentifrices. A mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an antiseptic wash containing sodium borate, glycerin and potassium bicarbonate. The active ingredient may also be dispersed in dentifrices, including: gels, pastes, powders and slurries. The active ingredient may be added in a therapeutically effective amount to a paste dentifrice that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants.

The compositions of the present invention may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for
example, "Remington's Pharmaceutical Sciences," 15th Ed., 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologies standards.

B. Bright/ARID2a Inhibitors

The present invention contemplates the use of any composition that will inhibit Bright/ARID3a function. Biological inhibitors, as described below, may be utilized to interfere with Bright/ARID3a function.

1. Antisense Constructs

Antisense methodology takes advantage of the fact that nucleic acids tend to pair with "complementary" sequences. By complementary, it is meant that polynucleotides are those which are capable of base-pairing according to the standard Watson-Crick complementarity rules. That is, the larger purines will base pair with the smaller pyrimidines to form combinations of guanine paired with cytosine (G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. Inclusion of less common bases such as inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others in hybridizing sequences does not interfere with pairing.

Targeting double-stranded (ds) DNA with polynucleotides leads to triple-helix formation; targeting RNA will lead to double-helix formation. Antisense polynucleotides, when introduced into a target cell, specifically bind to their target polynucleotide and interfere with transcription, RNA processing, transport, translation and/or stability. Antisense RNA constructs, or DNA encoding such antisense RNA's, may be employed to inhibit gene transcription or translation or both within a host cell, either in vitro or in vivo, such as within a host animal, including a human subject.

Antisense constructs may be designed to bind to the promoter and other control regions, exons, introns or even exon-intron boundaries of a gene. It is contemplated that the most effective antisense constructs will include regions complementary to intron/exon splice junctions. Thus, it is proposed that a preferred embodiment includes an antisense construct with complementarity to regions within 50-200 bases of an intron-exon splice junction. It has been observed that some exon sequences can be included in the construct without seriously
affecting the target selectivity thereof. The amount of exonic material included will vary depending on the particular exon and intron sequences used. One can readily test whether too much exon DNA is included simply by testing the constructs in vitro to determine whether normal cellular function is affected or whether the expression of related genes having complementary sequences is affected.

As stated above, "complementary" or "antisense" means polynucleotide sequences that are substantially complementary over their entire length and have very few base mismatches. For example, sequences of fifteen bases in length may be termed complementary when they have complementary nucleotides at thirteen or fourteen positions. Naturally, sequences which are completely complementary will be sequences which are entirely complementary throughout their entire length and have no base mismatches. Other sequences with lower degrees of homology also are contemplated. For example, an antisense construct which has limited regions of high homology, but also contains a non-homologous region (e.g., ribozyme; see below) could be designed. These molecules, though having less than 50% homology, would bind to target sequences under appropriate conditions.

It may be advantageous to combine portions of genomic DNA with cDNA or synthetic sequences to generate specific constructs. For example, where an intron is desired in the ultimate construct, a genomic clone will need to be used. The cDNA or a synthesized polynucleotide may provide more convenient restriction sites for the remaining portion of the construct and, therefore, would be used for the rest of the sequence.

2. Ribozymes

Another general class of inhibitors is ribozymes. Although proteins traditionally have been used for catalysis of nucleic acids, another class of macromolecules has emerged as useful in this endeavor. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclease activity (Kim and Cook, 1987; Gerlach et al., 1987; Forster and Symons, 1987). For example, a large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Cook et al., 1981; Michel and Westhof, 1990; Reinhold-Hurek and Shub, 1992). This specificity has been attributed to the requirement that the substrate bind via specific base-pairing interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.
Ribozyme catalysis has primarily been observed as part of sequence-specific cleavage/ligation reactions involving nucleic acids (Joyce, 1989; Cook et al, 1981). For example, U.S. Patent 5,354,855 reports that certain ribozymes can act as endonucleases with a sequence specificity greater than that of known ribonucleases and approaching that of the DNA restriction enzymes. Thus, sequence-specific ribozyme-mediated inhibition of gene expression may be particularly suited to therapeutic applications (Scanlon et al, 1991; Sarver et al, 1990). It has also been shown that ribozymes can elicit genetic changes in some cell lines to which they were applied; the altered genes included the oncogenes H-ras, c-fos and genes of HIV. Most of this work involved the modification of a target mRNA, based on a specific mutant codon that was cleaved by a specific ribozyme.

3. **RNAi**

RNA interference (also referred to as "RNA-mediated interference" or RNAi) is another mechanism by which protein expression can be reduced or eliminated. Double-stranded RNA (dsRNA) has been observed to mediate the reduction, which is a multi-step process. dsRNA activates post-transcriptional gene expression surveillance mechanisms that appear to function to defend cells from virus infection and transposon activity (Fire et al, 1998; Grishok et al, 2000; Ketting et al, 1999; Lin et al, 1999; Montgomery et al, 1998; Sharp et al, 2000; Tabara et al, 1999). Activation of these mechanisms targets mature, dsRNA-complementary mRNA for destruction. RNAi offers major experimental advantages for study of gene function. These advantages include a very high specificity, ease of movement across cell membranes, and prolonged down-regulation of the targeted gene (Fire et al, 1998; Grishok et al, 2000; Ketting et al, 1999; Lin et al, 1999; Montgomery et al, 1998; Sharp, 1999; Sharp et al, 2000; Tabara et al, 1999). Moreover, dsRNA has been shown to silence genes in a wide range of systems, including plants, protozoans, fungi, C. elegans, Trypanosoma, Drosophila, and mammals (Grishok et al, 2000; Sharp, 1999; Sharp et al, 2000; Elbashir et al, 2001). It is generally accepted that RNAi acts post-transcriptionally, targeting RNA transcripts for degradation, and possibly by inhibiting translation. It appears that both nuclear and cytoplasmic RNA can be targeted (Bosher et al, 2000).

siRNAs must be designed so that they are specific and effective in suppressing the expression of the genes of interest. Methods of selecting the target sequences, i.e. those sequences present in the gene or genes of interest to which the siRNAs will guide the degradative machinery, are directed to avoiding sequences that may interfere with the
siRNA's guide function while including sequences that are specific to the gene or genes. Typically, siRNA target sequences of about 21 to 23 nucleotides in length are most effective. This length reflects the lengths of digestion products resulting from the processing of much longer RNAs as described above (Montgomery et al., 1998). Of particular interest are those siRNAs that span an exon-intron junction.

The making of siRNAs has been mainly through direct chemical synthesis; through processing of longer, double stranded RNAs through exposure to Drosophila embryo lysates; or through an in vitro system derived from S2 cells. Use of cell lysates or in vitro processing may further involve the subsequent isolation of the short, 21-23 nucleotide siRNAs from the lysate, etc., making the process somewhat cumbersome and expensive. Chemical synthesis proceeds by making two single stranded RNA-oligomers followed by the annealing of the two single stranded oligomers into a double stranded RNA. Methods of chemical synthesis are diverse. Non-limiting examples are provided in U.S. Patents 5,889,136, 4,415,732, and 4,458,066, expressly incorporated herein by reference, and in Wincott et al. (1995).

Several further modifications to siRNA sequences have been suggested in order to alter their stability or improve their effectiveness. It is suggested that synthetic complementary 21-mer RNAs having di-nucleotide overhangs (i.e., 19 complementary nucleotides + 3’ non-complementary dimers) may provide the greatest level of suppression. These protocols primarily use a sequence of two (2’-deoxy) thymidine nucleotides as the di-nucleotide overhangs. These dinucleotide overhangs are often written as dTdT to distinguish them from the typical nucleotides incorporated into RNA. The literature has indicated that the use of dT overhangs is primarily motivated by the need to reduce the cost of the chemically synthesized RNAs. It is also suggested that the dTdT overhangs might be more stable than UU overhangs, though the data available shows only a slight (< 20%) improvement of the dTdT overhang compared to an siRNA with a UU overhang.

Chemically synthesized siRNAs are found to work optimally when they are in cell culture at concentrations of 25-100 nM. This had been demonstrated by Elbashir et al. (2001) wherein concentrations of about 100 nM achieved effective suppression of expression in mammalian cells. siRNAs have been most effective in mammalian cell culture at about 100 nM. In several instances, however, lower concentrations of chemically synthesized siRNA have been used (Caplen et al., 2000; Elbashir et al., 2001).

WO 99/32619 and WO 01/68836 suggest that RNA for use in siRNA may be chemically or enzymatically synthesized. Both of these texts are incorporated herein in their entirety by reference. The enzymatic synthesis contemplated in these references is by a
cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, SP6) via the use and production of an expression construct as is known in the art. See U.S. Patent 5,795,715. The contemplated constructs provide templates that produce RNAs that contain nucleotide sequences identical to a portion of the target gene. The length of identical sequences provided by these references is at least 25 bases, and may be as many as 400 or more bases in length. An important aspect of this reference is that the authors contemplate digesting longer dsRNAs to 21-25mer lengths with the endogenous nuclease complex that converts long dsRNAs to siRNAs in vivo. They do not describe or present data for synthesizing and using in vitro transcribed 21-25mer dsRNAs. No distinction is made between the expected properties of chemical or enzymatically synthesized dsRNA in its use in RNA interference.

Similarly, WO 00/44914, incorporated herein by reference, suggests that single strands of RNA can be produced enzymatically or by partial/total organic synthesis. Preferably, single stranded RNA is enzymatically synthesized from the PCR™ products of a DNA template, preferably a cloned cDNA template and the RNA product is a complete transcript of the cDNA, which may comprise hundreds of nucleotides. WO 01/36646, incorporated herein by reference, places no limitation upon the manner in which the siRNA is synthesized, providing that the RNA may be synthesized in vitro or in vivo, using manual and/or automated procedures. This reference also provides that in vitro synthesis may be chemical or enzymatic, for example using cloned RNA polymerase (e.g., T3, T7, SP6) for transcription of the endogenous DNA (or cDNA) template, or a mixture of both. Again, no distinction in the desirable properties for use in RNA interference is made between chemically or enzymatically synthesized siRNA.

U.S. Patent 5,795,715 reports the simultaneous transcription of two complementary DNA sequence strands in a single reaction mixture, wherein the two transcripts are immediately hybridized. The templates used are preferably of between 40 and 100 base pairs, and which is equipped at each end with a promoter sequence. The templates can be attached to a solid surface. After transcription with RNA polymerase, the resulting dsRNA fragments may be used for detecting and/or assaying nucleic acid target sequences.

In a specific embodiment, the inventor proposes to inhibit ARID3a expression in adult tissues in vitro using siRNA or shRNA in a lentiviral vector. A GFP marker can be utilized to determine that cells took up the vector, and thus permit checking for appropriate inhibition of ARID3a production. The B cell line BCg3R-ld and/or over-expressing transgenic mouse spleen cells can be utilized. After the inventor confirms that inhibition of ARID3a occurs in these cells, they will inhibit ARID3a expression in mouse embryo fibroblasts and culture the
GFP+ cells to confirm that pluripotent stem cells develop. The use of an inducible promoter (discussed below) that allow induction of the siRNA or shRNA only under specific growth conditions permit reversible inhibition of ARID3a. Thus, cells can be induced to dedifferentiate into a pluripotent and self-renewing state in vitro, and can then be induced to differentiate into mature lineage cells under different growth conditions without inhibition of ARID3a. These methods offer considerable advantages over current methodologies, which involve introduction of multiple viral copies and genes, some of which are known to be oncogenic. Self-deleting vectors may also be used.

4. Antibodies

In certain aspects of the invention, antibodies may find use as inhibitors of ARID3a. As used herein, the term "antibody" is intended to refer broadly to any appropriate immunologic binding agent such as IgG, IgM, IgA, IgD and IgE. Generally, IgG and/or IgM are preferred because they are the most common antibodies in the physiological situation and because they are most easily made in a laboratory setting.

The term "antibody" also refers to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')2, single domain antibodies (DABs), Fv, scFv (single chain Fv), and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art.

Monoclonal antibodies (MAbs) are recognized to have certain advantages, e.g., reproducibility and large-scale production, and their use is generally preferred. The invention thus provides monoclonal antibodies of the human, murine, monkey, rat, hamster, rabbit and even chicken origin. Due to the ease of preparation and ready availability of reagents, murine monoclonal antibodies will often be preferred. Single-chain Mabs are described in U.S. Patents 4,946,778 and 5,888,773, each of which are hereby incorporated by reference. The present invention would most likely utilize single-chain antibodies expressed from expression vectors, as described below.

"Humanized" antibodies are also contemplated, as are chimeric antibodies from mouse, rat, or other species, bearing human constant and/or variable region domains, bispecific antibodies, recombinant and engineered antibodies and fragments thereof. Methods for the development of antibodies that are "custom-tailored" to the patient's dental disease are likewise known and such custom-tailored antibodies are also contemplated.
5. Inhibitory Peptides

Peptides may prove to be useful inhibitors of Bright/ARID3a function by competing with or mimicking Bright domains that bind or interact with DNA, Btk, TFII-I or other molecules, or compete with Bright dimerization sequences. Also contemplated are regions of Bright that comprise nuclear shuttling sequences. Bright-derived peptides are therefore a particular type of compound that may prove useful in inhibiting Bright function. The peptides may be designed around an existing structure, i.e., portions of Bright, or they may be selected for function from a randomized library.

Of particular interest is a region of SEQ ID NO:1 from about residue 444 to residue 549, and more particularly from 449-544. This region has been shown to be involved in Bright/ARID3a dimerization, and also to contain nuclear shuttling sequences. Within these regions, all possible peptides of 8 to about 40 residues are contemplated. Other more particular regions include residues 444-483, 449-488, 510-549, 505-544, 444-473, 449-473, 531-549 and 531-544.

In general, the peptides will be less than 50 residues, and comprising at least about 10 consecutive residues of Bright/ARID3a. The number of consecutive Bright/ARID3a residues may be 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, with additional non-Bright residues attached thereto. Ranges of peptide length of 10-50 residues, 10-40 residues, 15-50 residues 15-40, residues, 15-35 residues, 15-30 residues, 15-25 residues, 15-20 residues and 20-25 residues are contemplated. The number of additional non-Bright/ARID3a residues may be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more residues. The overall size of the peptides may therefore range from 8 residues to 75 or more residues, with 10-70 residues, 10-60 residues, 10-50 residues, 10-40 residues, 10-30 residues and 15-70 residues, 15-60 residues, 15-50 residues, 15-40 residues, 15-30 residues, 20-70 residues, 20-60 residues, 20-50 residues, 20-40 residues, and 20-30 residues being specifically contemplated ranges.

Peptides may be produced by cleavage of polypeptides, such as Bright, with proteolytic enzymes (trypsin, chymotrypsin, etc.), or chemicals. They may also be produced recombinantly using vectors and techniques described supra. However, it may be most advantageous to produce peptides using the solid-phase synthetic techniques (Merrifield, 1963). Other peptide synthesis techniques are well known to those of skill in the art (Bodanszky et al, 1976; Peptide Synthesis, 1985; Solid Phase Peptide Synthelia, 1984). Appropriate protective groups for use in such syntheses will be found in the above texts, as
well as in Protective Groups in Organic Chemistry, 1973. These synthetic methods involve
the sequential addition of one or more amino acid residues or suitable protected amino acid
residues to a growing peptide chain. Normally, either the amino or carboxyl group of the first
amino acid residue is protected by a suitable, selectively removable protecting group. A
different, selectively removable protecting group is utilized for amino acids containing a
reactive side group, such as lysine.

Using solid phase synthesis as an example, the protected or derivatized amino acid is
attached to an inert solid support through its unprotected carboxyl or amino group. The
protecting group of the amino or carboxyl group is then selectively removed and the next
amino acid in the sequence having the complementary (amino or carboxyl) group suitably
protected is admixed and reacted with the residue already attached to the solid support. The
protecting group of the amino or carboxyl group is then removed from this newly added
amino acid residue, and the next amino acid (suitably protected) is then added, and so forth.
After all the desired amino acids have been linked in the proper sequence, any remaining
terminal and side group protecting groups (and solid support) are removed sequentially or
concurrently, to provide the final peptide. The peptides of the invention are preferably
devoid of benzylated or methylbenzylated amino acids. Such protecting group moieties may
be used in the course of synthesis, but they are removed before the peptides are used.
Additional reactions may be necessary, as described elsewhere, to form intramolecular
linkages to restrain conformation.

Aside from the twenty standard amino acids can be used, there are a vast number
of "non-standard" amino acids. Two of these can be specified by the genetic code, but are
rather rare in proteins. Selenocysteine is incorporated into some proteins at a UGA codon,
which is normally a stop codon. Pyrrolysin is used by some methanogenic archaea in
enzymes that they use to produce methane. It is coded for with the codon UAG. Examples of
non-standard amino acids that are not found in proteins include lanthionine, 2-
aminoisobutyric acid, dehydroalanine and the neurotransmitter gamma-aminobutyric acid.
Non-standard amino acids often occur as intermediates in the metabolic pathways for
standard amino acids - for example ornithine and citrulline occur in the urea cycle, part of
amino acid catabolism. Non-standard amino acids are usually formed through modifications
to standard amino acids. For example, homocysteine is formed through the transsulfuration
pathway or by the demethylation of methionine via the intermediate metabolite S-adenosyl
methionine, while hydroxyproline is made by a posttranslational modification of proline.
The present invention may utilize L-configuration amino acids, D-configuration amino acids, or a mixture thereof. While L-amino acids represent the vast majority of amino acids found in proteins, D-amino acids are found in some proteins produced by exotic sea-dwelling organisms, such as cone snails. They are also abundant components of the peptidoglycan cell walls of bacteria. D-serine may act as a neurotransmitter in the brain. The L and D convention for amino acid configuration refers not to the optical activity of the amino acid itself, but rather to the optical activity of the isomer of glyceraldehyde from which that amino acid can theoretically be synthesized (D-glyceraldehyde is dextrorotary; L-glyceraldehyde is levorotary).

One form of an "all-D" peptide is a retro-inverso peptide. Retro-inverso modification of naturally occurring polypeptides involves the synthetic assemblage of amino acids with a-carbon stereochemistry opposite to that of the corresponding L-amino acids, i.e., D-amino acids in reverse order with respect to the native peptide sequence. A retro-inverso analogue thus has reversed termini and reversed direction of peptide bonds (NH-CO rather than CO-NH) while approximately maintaining the topology of the side chains as in the native peptide sequence. See U.S. Patent 6,261,569, incorporated herein by reference.

Peptides may advantageously be attached or fused to certain additional peptide segments for beneficial properties associated therewith. In particular, such domains are cell delivery domains (also called a cell delivery vector, or cell transduction domain). These types of domains are well known in the art and are generally characterized as short amphipathic or cationic peptides and peptide derivatives, often containing multiple lysine and arginine residues. Of particular interest are poly-D-Arg and poly-D-Lys sequences (e.g., dextrorotary residues, eight residues in length). Others are listed below in Table A.

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</tbody>
</table>
Linkers or cross-linking agents may be used to fuse peptides to other proteinaceous sequences. Bifunctional cross-linking reagents have been extensively used for a variety of purposes including preparation of affinity matrices, modification and stabilization of diverse structures, identification of ligand and receptor binding sites, and structural studies. Homobifunctional reagents that carry two identical functional groups proved to be highly efficient in inducing cross-linking between identical and different macromolecules or subunits of a macromolecule, and linking of polypeptide ligands to their specific binding sites. Heterobifunctional reagents contain two different functional groups. By taking advantage of the differential reactivities of the two different functional groups, cross-linking
can be controlled both selectively and sequentially. The bifunctional cross-linking reagents
can be divided according to the specificity of their functional groups, *e.g.*, amino, sulphhydryl,
guanidino, indole, carboxyl specific groups. Of these, reagents directed to free amino groups
have become especially popular because of their commercial availability, ease of synthesis
and the mild reaction conditions under which they can be applied. A majority of
heterobifunctional cross-linking reagents contains a primary amine-reactive group and a
thiol-reactive group.

In another example, heterobifunctional cross-linking reagents and methods of using
the cross-linking reagents are described in U.S. Patent 5,889,155, specifically incorporated
herein by reference in its entirety. The cross-linking reagents combine a nucleophilic
hydrazide residue with an electrophilic maleimide residue, allowing coupling in one example,
of aldehydes to free thiols. The cross-linking reagent can be modified to cross-link various
functional groups and is thus useful for cross-linking polypeptides. In instances where a
particular peptide does not contain a residue amenable for a given cross-linking reagent in its
native sequence, conservative genetic or synthetic amino acid changes in the primary
sequence can be utilized.

Peptides modified for *in vivo* use by the addition, at the amino- and/or carboxyl-
terminus ends, of a blocking agent to facilitate survival of the peptide *in vivo* are
contemplated. This can be useful in those situations in which the peptide termini tend to be
degraded by proteases prior to cellular uptake. Such blocking agents can include, without
limitation, additional related or unrelated peptide sequences that can be attached to the amino
and/or carboxyl terminal residues of the peptide to be administered. These agents can be
added either chemically during the synthesis of the peptide, or by recombinant DNA
technology by methods familiar in the art. Alternatively, blocking agents such as
pyroglutamic acid or other molecules known in the art can be attached to the amino and/or
carboxyl terminal residues.

The inventor also contemplated that certain non-natural amino acids that satisfy the
structural constraints of the inhibitory peptides of the present invention without a loss, and
perhaps with an improvement in, biological function. In addition, the present inventor also
contemplated that structurally similar compounds may be formulated to mimic the key
portions of Bright/ARTD3a. Such compounds, which may be termed peptidomimetics, may
be used in the same manner as the peptides of the invention and, hence, also are functional
equivalents.
Certain mimetics that mimic elements of protein secondary and tertiary structure are described in Johnson et al. (1993). The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and/or antigen. A peptide mimetic is thus designed to permit molecular interactions similar to the natural molecule.

Methods for generating specific structures have been disclosed in the art. For example, α-helix mimetics are disclosed in U.S. Patents 5,446,128; 5,710,245; 5,840,833; and 5,859,184. Methods for generating conformationally restricted β-turns and β-bulges are described, for example, in U.S. Patents 5,440,013; 5,618,914; and 5,670,155. Other types of mimetic turns include reverse and γ-turns. Reverse turn mimetics are disclosed in U.S. Patents 5,475,085 and 5,929,237, and γ-turn mimetics are described in U.S. Patents 5,672,681 and 5,674,976.

By "molecular modeling" is meant quantitative and/or qualitative analysis of the structure and function of protein-protein physical interaction based on three-dimensional structural information and protein-protein interaction models. This includes conventional numeric-based molecular dynamic and energy minimization models, interactive computer graphic models, modified molecular mechanics models, distance geometry and other structure-based constraint models. Molecular modeling typically is performed using a computer and may be further optimized using known methods. Computer programs that use X-ray crystallography data are particularly useful for designing such compounds. Programs such as RasMol, for example, can be used to generate three dimensional models. Computer programs such as INSIGHT (Accelrys, Burlington, MA), GRASP (Anthony Nicholls, Columbia University), Dock (Molecular Design Institute, University of California at San Francisco), and Auto-Dock (Accelrys) allow for further manipulation and the ability to introduce new structures. The methods can involve the additional step of outputting to an output device a model of the 3-D structure of the compound. In addition, the 3-D data of candidate compounds can be compared to a computer database of, for example, 3-D structures.

Compounds of the invention also may be interactively designed from structural information of the compounds described herein using other structure-based design/modeling techniques (see, e.g., Jackson, 1997; Jones et al., 1996). Candidate compounds can then be
tested in standard assays familiar to those skilled in the art. Exemplary assays are described herein.

The 3-D structure of biological macromolecules (e.g., proteins, nucleic acids, carbohydrates, and lipids) can be determined from data obtained by a variety of methodologies. These methodologies, which have been applied most effectively to the assessment of the 3-D structure of proteins, include: (a) x-ray crystallography; (b) nuclear magnetic resonance (NMR) spectroscopy; (c) analysis of physical distance constraints formed between defined sites on a macromolecule, e.g., intramolecular chemical crosslinks between residues on a protein (e.g., PCT/US00/14667, the disclosure of which is incorporated herein by reference in its entirety), and (d) molecular modeling methods based on a knowledge of the primary structure of a protein of interest, e.g., homology modeling techniques, threading algorithms, or ab initio structure modeling using computer programs such as MONSSTER (Modeling Of New Structures from Secondary and Tertiary Restraints) (see, e.g., International Application No. PCT/US99/11913, the disclosure of which is incorporated herein by reference in its entirety). Other molecular modeling techniques may also be employed in accordance with this invention (e.g., Cohen et al., 1990; Navia et al., 1992), the disclosures of which are incorporated herein by reference in their entirety). All these methods produce data that are amenable to computer analysis. Other spectroscopic methods that can also be useful in the method of the invention, but that do not currently provide atomic level structural detail about biomolecules, include circular dichroism and fluorescence and ultraviolet/visible light absorbance spectroscopy. A particular method of analysis is x-ray crystallography.

6. Dominant-Negative Bright/ARID3a

Dominant-negative proteins are defective proteins with can negate the effects of normal, functional proteins when both are present in the same environment. In many cases, dominant-negative proteins homo-multimerize and are thus able to "poison" a complex that contains one or more functional proteins. Dominant-negative forms of Bright have been produced which act in just this manner. In designing dominant-negative Bright molecules, several regions present useful points for mutation. First, changes in the DNA binding domain (ARID) that block DNA binding produce dominant-negative effects. Second, alterations in the nuclear localization sequence which block nuclear translocation result in a dominant-negative form of Bright/ARID3a. Third, manipulation of the interaction and dimerization domains causes a dominant-negative function. Other dominant-negative proteins may be
produced by interfering with the amino-terminal domain. Dominant-negative forms of Bright are described in Nixon et al. (2004a; 2004b).

VIII. Kits

For use in the applications described herein, kits are also within the scope of the invention. Such kits can comprise a carrier, package or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in the method, in particular, a Bright inhibitor. The kit of the invention will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. In addition, a label can be provided on the container to indicate that the composition is used for a specific therapeutic application, and can also indicate directions for either in vivo or in vitro use, such as those described above. Directions and or other information can also be included on an insert which is included with the kit. In particular, kits according to the present invention contemplate the assemblage of agents for assessing ARID3a levels along with one or more of an SLE therapeutic and/or a reagent for assessing antinuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA), as well as controls for assessing the same.

IX. Examples

The following examples are included to further illustrate various aspects of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques and/or compositions discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1 - MATERIALS AND METHODS

Participants. Healthy age and gender-matched controls and patients who met a minimum of four American College of Rheumatology Classification Criteria for SLE and for seropositive rheumatoid arthritis (RA) were recruited after informed consent from the
Oklahoma Medical Research Foundation Clinical Pharmacology clinic at as part of the Oklahoma Lupus Cohort (IRB compliance #09-07 and #06-19), in accordance with the Declaration of Helsinki. Peripheral blood mononuclear cells from a total of 115 SLE patients (ranging in age from 21 to 72, 94% female), 6 RA patients and 33 healthy controls were analyzed for ARID3a expression. Forty-four of the 115 SLE patients provided longitudinal samples at 2-3 visits (mean 2.6) over a 36 month period. SLE patients included 42 women and 2 men ranging from 21 to 66 years of age. Age at diagnosis and first blood draw, ethnic background and immunosuppressive medications taken at the first blood draw are given online in Table 1:

### Table 1 - SLE Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, no.</td>
<td>44</td>
</tr>
<tr>
<td>Female, iss. (%)</td>
<td>42 (95)</td>
</tr>
<tr>
<td>Caucasian, nr. (%)</td>
<td>17 (33)</td>
</tr>
<tr>
<td>Caucasian/Hispanic, no. (%)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>African, Is. (%)</td>
<td>16 (33)</td>
</tr>
<tr>
<td>American Indian, no. (%)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Asian, no. (%)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Age, years</td>
<td>40 (21-66)</td>
</tr>
<tr>
<td>Age at diagnosis, years</td>
<td>31 (13-61)</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>5 (0-15)</td>
</tr>
<tr>
<td>Immunosuppressive medications, no. (%)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>7 (16)</td>
</tr>
<tr>
<td>Belimumab</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>12 (27)</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>29 (54)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>12 (27)</td>
</tr>
<tr>
<td>Prednisone</td>
<td>16 (36)</td>
</tr>
</tbody>
</table>

*Except when indicated, values represent the mean (range). SLE = systemic lupus erythematosus.

Four patients were not taking medication at their first blood draw. Further details of RA patient characteristics can be found online in Table 2:
Table 2 - RA Patient Characteristics

<table>
<thead>
<tr>
<th>Patients, no.</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, no. (%)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Caucasian, %</td>
<td>4 (66)</td>
</tr>
<tr>
<td>Caucasian/American Indian, %</td>
<td>1 (17)</td>
</tr>
<tr>
<td>American Indian, no. (%)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Age, years</td>
<td>57 (46-70)</td>
</tr>
<tr>
<td>DAS2S</td>
<td>2.2 (1.61-3.95)</td>
</tr>
</tbody>
</table>

Immune modulatory medications, no. (%)

<table>
<thead>
<tr>
<th>Medication</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>LeSomnoids</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Etanercept</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Abatacept</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Tofacitinib citrate</td>
<td>1 (17)</td>
</tr>
</tbody>
</table>

*Except when indicated, values represent the mean (range).

RA = rheumatoid arthritis; DAS28 = Disease activity index

Flow Cytometry. Mononuclear cells were isolated from heparinized peripheral blood (~15 ml) with Ficoll-Paque Plus (GE Healthcare) and stained with the following fluorochrome-labeled antibodies: CD19 PE-Cy5, CD24 APC, IL-10 PE, CD10 Pacific Blue (BioLegend), IgD PerCP-Cy5.5, CD27 PE-Cy7, CD3 Pacific Blue (BD Biosciences), CD38 Alexa Fluor 700 (BD Pharmingen), and IgM APC (Southern Biotech). Cells were fixed with 2% paraformaldehyde, permeabilized with 0.1% Tween-20 and stained with goat anti-human ARID3a antibody (14) followed by rabbit anti-goat IgG FITC (Invitrogen). Gating for individual B cell subsets is shown in FIG. 6 as described (15,16). Isotype controls (Caltag, BD Pharmingen, and eBioscience) were used for gating. Data (500,000 events per sample) were collected using an LSRII (BD Biogenics) and FACSDiva (BD Biosciences) software.
version 4.1 and analyzed using CellQuest Pro (BD Biosciences) and FlowJo (Tree Star) software versions 6.0 and 9.5.2, respectively.

**Western Blots and ELISAs.** Western blots for ARID3a were performed as previously described (17) using 20,000 peripheral blood mononuclear cells per lane. β-actin (Santa Cruz Biotechnology) was detected as a loading control. Immunoglobulin levels were determined from serially diluted plasma samples using alkaline phosphatase-conjugated anti-human IgG and IgM (Southern Biotech) and a Biotech EL800 plate reader at 405/490nm with GEN5 Biotech software. Autoantibody testing was determined using the Reichlin method as described (18). Levels of plasma TGF-β, IL-17, IL-10, TNFa and MIP1α were detected using a Single-Analyte ELISArray Kit (QIAGEN) according to the manufacturer’s directions.

**Statistics.** Data were statistically evaluated using Students’ t test or Mann Whitney U tests with (GraphPad) Prism software version 6.0, as indicated. P values of less than 0.05 were considered significant. Association of SLEDAI scores from each visit for each SLE patient with numbers of total ARID3+B cells at that time, and with numbers of ARID3a+B cells of each B cell subset, were performed by best fit linear regression models using the linear models function in R version 3.0.0 (within a Linux operating system). A stepwise modeling approach was used wherein all initial, fully saturated models included adjustment for sex, age, race and overall B cell count. Variables that did not significantly contribute to the model fit (i.e., whose p-value was > 0.05) were removed. The final and most parsimonious models for each of the subsets adjusted only for overall B cell counts since the other covariates (sex, age and race) were found to be noncontributory based on the above criteria.

**EXAMPLE 2 - RESULTS**

A flow cytometry assay for intracellular ARID3a was developed, validated and used to assess expression in SLE patient B cells. Mononuclear peripheral blood cells from SLE patients and healthy controls were gated for B (CD19+) or T (CD3+) lymphocyte surface markers (FIG. 1A). As expected, healthy control samples contained very low numbers of B cells expressing intracellular ARID3a (< 15%). ARID3a expression in T cells was not above background in either control or SLE patients. However, numbers of ARID3a+B cells were either greatly increased (50 of 115 patients) in a cross section of lupus patient samples, or were similar to healthy controls (e.g., FIG. 1A, SLE # 2 vs SLE#15). Western blotting confirmed that intracellular staining represented ARID3a protein expression and not an
unidentified cross-reactivity; patient samples with abundant numbers of ARID3a+ B cells also showed abundant ARID3a protein (FIG. 1B). At their first visit, 45% of SLE patients in the inventor’s longitudinal study (19 of 42) showed increased numbers of ARID3a+ B cells more than two standard deviations above the mean of the healthy controls (FIG. 1C, open circles). The inventor defined those samples as ARID3aH (high), while SLE patient samples with ARID3a+ cells within healthy control ranges were designated ARID3aN (normal). Numbers of ARID3a+ CD19+ B cells in ARID3aH patients ranged from 3- to > 40-fold higher than average numbers of ARID3a+ cells in controls or ARID3aN patients. RA patient samples resembled healthy controls and did not show increased numbers of ARID3a+ B cells. These data indicate that ARID3a+ B cell numbers are dramatically expanded in a large subset of SLE patients.

Disease activity in patients was determined using the SLE Disease Activity Index (SLEDAI) score (19) and spanned a wide range of lupus activity (scores of 0-15). Average SLEDAI scores of patients having ARID3aH samples at the first visit (FIG. 1D) were significantly higher than the SLEDAI scores of patients with ARID3aN samples (p = 0.017). No correlations between ARID3a expression and age, race or duration of disease were observed. Likewise, no correlations were observed between numbers of ARID3a+ B cells and medications in use (Table 3). Two of four samples designated as ARID3aH were from patients taking no immune-modulatory medications at the first visit. These data suggest a possible correlation with disease activity and numbers of ARID3a+ B cells.

**Table 3 - Medications taken at first visit do not correlate with ARID3a expression**

<table>
<thead>
<tr>
<th>ARID3aN (23)</th>
<th>Hydroxychloroquine</th>
<th>Deferasirox</th>
<th>Pirenidone*</th>
<th>Azathioprine</th>
<th>Mycophenolate</th>
<th>Rituximab</th>
<th>Belimumab</th>
<th>Methotrexate</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 (65%)</td>
<td>7 (30%)</td>
<td>10 (40%)</td>
<td>2 (9%)</td>
<td>8 (35%)</td>
<td>1 (4%)</td>
<td>1 (8%)</td>
<td>4 (24%)</td>
<td></td>
</tr>
<tr>
<td>ARID3aH (4)</td>
<td>14 (82%)</td>
<td>5 (29%)</td>
<td>6 (38%)</td>
<td>5 (29%)</td>
<td>6 (35%)</td>
<td>0 (0%)</td>
<td>4 (24%)</td>
<td></td>
</tr>
</tbody>
</table>

* - Four patients were taking no immunomodulatory medications

The inventor’s previous analyses of human B lineage subpopulations indicated that ARID3a mRNA transcripts were predominantly limited to pre-B, antibody-secreting and memory B cell subpopulations, but were not detectable in immature or naive mature B cells in healthy individuals (14). Because circulating B cell subpopulations in SLE patients may be skewed compared to healthy populations (20), the inventor examined the possibility that
increased numbers of ARID3a+ cells might reflect disproportionate numbers of memory B or
plasma cells in the ARID3aH versus the ARID3aN samples. B cell numbers were quantified
within eight different B cell subpopulations (gated as in FIG. 6) from ARID3aN and ARID3aH
samples (FIG. 7) by flow cytometry. Although average numbers of total B cells were slightly
increased in the ARID3aH compared to the ARID3aN patient samples, they did not differ
significantly from those of healthy controls. In addition, average numbers of most B cell
subtypes were similar between control and ARID3aH samples. Therefore, increased numbers
of ARID3a+ B cells in the ARID3aH patient samples are not the result of global expansion of
all B cells, nor could they be attributed to expansion of B cell subpopulations that normally
express ARID3a.

The inventor next compared ARID3a expression levels within each B cell subset from
SLE and RA patients to healthy controls (FIGS. 2A-B) to determine which subset(s)
accounted for the increased expression observed in FIG. 1C. In healthy controls, ARID3a
expression was observed in very small numbers of naïve and transitional (IgM+IgD+CD27−) B
cells, but was more evident in the CD27+ memory B cell populations and
plasmablasts/plasma cells (FIG. 2A, top panels). ARID3a expression in B cell subsets from
ARID3aN samples (FIG. 2A, middle panels) closely resembled those of healthy controls.
However, ARID3aH samples (FIG. 2A, bottom panels) typically showed increased numbers
of ARID3a-expressing cells in all subpopulations compared to both healthy control and
ARID3aN samples. Unexpectedly, two distinct peaks were evident in many B cell subsets
from ARID3aH samples, indicating that only some cells expressed ARID3a while others did
not. Enumeration of ARID3+ B cells in each subset (FIG. 2B) indicated that some SLE
samples contained more than 100 times the numbers of ARID3a+ naïve B cells found in
average healthy control or RA patient samples. Numbers of ARID3a+ memory IgM, double
negative (DN) B cells and plasmablasts/ plasma cells were also significantly increased
(p<0.05) in SLE patients compared to healthy control and RA samples. The inventor
previously showed that resting naive B cells from healthy controls do not transcribe
detectable levels of ARID3a (14). However, immature transitional B cells from which the
naïve B cells develop, expressed ARID3a in mice (21), and these cells were included within
the naïve subpopulation gate. Therefore, this B cell subset was further segregated using CD10
to distinguish transitional cells from naïve B cells. ARID3a+ B cells occurred in both naïve
and transitional subsets (FIGS. 8A-B), indicating that increased numbers of ARID3a+
transitional B cells within the IgM+IgD+CD27− subset did not account for increases in
ARID3a+ naïve B cells in ARID3aH patient samples. These data demonstrate that ARID3a
expression in SLE patients can occur in any B cell subset, and that numbers of ARID3a expressing naive B cells are highly increased in some patients.

To determine if increased ARID3a expression was a stable phenotype in individual patients over time, patients were divided into ARID3a\(^{H}\) and ARID3a\(^{N}\) subsets based on analyses of CD19\(^{+}\) ARID3a\(^{+}\) B cell numbers during their first visit and ARID3a\(^{+}\) B cell numbers were plotted for subsequent visits (FIG. 3). Ten of 15 ARID3a\(^{H}\) patients showed normal numbers of ARID3a\(^{+}\) cells on at least one visit, and several of those were designated ARID3a\(^{N}\) on both of their subsequent visits. Eight of 22 patients with normal numbers of ARID3a\(^{+}\) B cells at their first visit showed increased numbers of ARID3a\(^{+}\) cells at a single subsequent visit. Percentages of ARID3a\(^{+}\) cells within individual B cell subsets also varied over time in all individuals (not shown). No correlations between alterations in immunosuppressive drugs and ARID3a\(^{+}\) B cell numbers were observed. Healthy controls were also assessed at multiple time points, and with two exceptions where individuals had recent viral infections, numbers of ARID3a\(^{+}\) B cells varied less than one standard deviation from the mean. Thus, numbers of ARID3a\(^{+}\) B cells in SLE patients vary over time, and individual patients may cycle between different ARID3a phenotypes including periods more consistent with healthy controls.

Higher SLEDAI scores were observed in patients whose samples were categorized as ARID3a\(^{H}\) on their first visit (FIG. ID). Using data from every patient at all visits, the inventor asked if lupus disease activity correlated with total numbers of ARID3a\(^{+}\) B cells and/or numbers of ARID3a\(^{+}\) B cells in any B cell subset. Associations between ARID3a\(^{+}\) cell numbers for each B cell subset and SLEDAI scores were explored at each visit, adjusting for gender, age, race and total B cell counts. Increased disease activity (SLEDAI scores) is associated with increased total numbers of circulating CD19\(^{+}\) ARID3a\(^{+}\) cells (Table 1, p=0.0039; and FIG. 9, Spearman’s correlation, p=0.006) and with numbers of ARID3a\(^{+}\) MZ memory (p=0.0225) and plasmablast/ plasma cells (p=0.0474), but not with the high numbers of naive ARID3a\(^{+}\) B cells (FIG. 2B and Table 4). These data suggest that increased numbers of mature ARID3a\(^{+}\) B cells within these specific B cell subsets are preferentially associated with increased disease activity in SLE. Moreover, alterations in ARID3a expression were associated with changes in disease activity in all patients regardless of segregation into the ARID3a\(^{H}\) or ARID3a\(^{N}\) SLE phenotype.
Table 4 - Relationship between ARID3a+ cells with SLEDAI for each B cell subset

<table>
<thead>
<tr>
<th>B Cell Subset</th>
<th>Coef. Est</th>
<th>Std. Error</th>
<th>P_pfrsimov *</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARID3a CD19</td>
<td>0.06624</td>
<td>0.0235 1</td>
<td>0.0039 **</td>
</tr>
<tr>
<td>ARID3a MZ Memory</td>
<td>0.8572 1</td>
<td>0.35807 0</td>
<td>0.0225 *</td>
</tr>
<tr>
<td>ARID3a Plasmablasts</td>
<td>5.32660</td>
<td>2.64755 2</td>
<td>0.0474 *</td>
</tr>
<tr>
<td>ARID3a Class-switched Memory</td>
<td>0.72306</td>
<td>0.36045 0</td>
<td>0.0514</td>
</tr>
<tr>
<td>ARID3a Naive</td>
<td>0.15942</td>
<td>0.09129 0</td>
<td>0.0862</td>
</tr>
<tr>
<td>ARID3a DN Memory</td>
<td>1.34904</td>
<td>0.79118 0</td>
<td>0.0990</td>
</tr>
<tr>
<td>ARID3a Memory IgM</td>
<td>0.16245</td>
<td>0.11892 0</td>
<td>0.1180</td>
</tr>
<tr>
<td>ARID3a C delta Class-switched</td>
<td>1.18269</td>
<td>0.80329 0</td>
<td>0.1232</td>
</tr>
<tr>
<td>ARID3a Naive IgD</td>
<td>0.27813</td>
<td>0.33544 0</td>
<td>0.3886</td>
</tr>
</tbody>
</table>

(*) P<0.05, (**) P<0.01; # P-value for the most parsimonious model. All models were adjusted for the total B cell counts for each B cell subset.

To further explore the basis for the association of ARID3a expression with disease activity, the inventor examined plasma Ig levels. Although ARID3aH samples had increased plasma IgM and IgG levels compared to healthy control and ARID3aN samples (FIG. 10A), and there was little difference in reactivity of the plasma with a large number of common autoantigens (FIG. 10B). These data suggest that increased numbers of total ARID3a+ peripheral blood B cells do not correlate with autoantibody reactivity patterns.

To determine whether ARID3a expression correlated with B cell activation status, the inventor explored expression of the activation marker CD86 and the pre-plasmablast marker CD43 (22-24). Naive B cells are enriched in the CD27- subset which had larger numbers of ARID3a− cells in many SLE patients, as shown in FIG. 2B. Therefore, the inventor examined ARID3a expression in both CD27-CD86+ and CD27-CD43+ B cell subpopulations of SLE, healthy control and RA patient samples (FIG. 4A). As expected, a portion of activated CD27- B cells expressed ARID3a (< 30%), with larger percentages occurring in both SLE and RA patient samples compared to healthy controls. However, ARID3a+ cells did not segregate with these markers. The majority of CD27-ARID3a+ SLE B cells (> 56%) did not express CD86 or CD43 (FIG. 4B). Therefore, the inventor concluded that ARID3a expression does not correlate with activation status as defined by expression of CD86, or with CD43 expression.
The inventor next evaluated inflammatory cytokine levels previously associated with increased disease severity in SLE. TGF-β, IL-17 (FIG. 5) TNF-α and MIPIα levels (not shown) were similar in ARID3aH and ARID3aN samples. Mean IL-10 levels were higher in patients than in healthy controls, with even greater increases in ARID3aH samples (FIG. 5).

Numbers of IL-10 secreting BIO B cells that act as negative regulators of the immune response (25) were also examined (not shown). No differences in BIO cell numbers between ARID3aH and ARID3aN SLE patients were observed, suggesting that non-circulating B cells or other cell types are responsible for increased IL-10 production in SLE patients.

EXAMPLE 3 - DISCUSSION

A cross-sectional analysis of 115 samples revealed increased numbers of ARID3a+ peripheral blood B lymphocytes in 43% of lupus patients. ARID3a expression was not limited to B cell differentiation stages that express ARID3a in healthy individuals, but occurred in all patient B cell subsets examined. Total numbers of ARID3a+ B cells in 44 patients assessed at multiple time points varied over time, and showed a strong association with SLEDAI scores at each visit. Increased disease activity correlated with total numbers of ARID3a+ B cells and with ARID3a+ marginal zone memory and plasmablast/plasma cells. These data suggest that numbers of ARID3a+ B cells might be tested as a biomarker to stratify patients in studies of pathogenic mechanisms and/or future trials of relevant targeted treatments.

Subdivision of these SLE patients based on ARID3a expression in B cells did not reveal associations with demographics or age. Two patients receiving no immunosuppressive medications over-expressed ARID3a suggesting that increased numbers of ARID3a+ B cells were not the result of treatment. Rather, ARID3a expression was strongly associated with disease activity. Because total numbers of ARID3a+ cells/ml were used to define ARID3a over-expression, and many patients received immunomodulatory medications during these studies, it is possible that the degree of association between ARID3a expression and disease activity may be an underestimation.

Although ARID3a/Bright enhances transcription of some Ig heavy chains (17), recent data suggest it can also repress transcription of other genes (26,27). Many ARID family proteins are members of large epigenetic regulatory complexes (28), although it is not clear if ARID3a serves similar types of functions in B lymphocytes. Perhaps the most surprising finding was that ARID3aH patient samples displayed bimodal ARID3a expression in most B cell subsets. Because the inventor's data show strong associations between numbers of
ARID3a<sup>+</sup> B cells and disease activity, it will be important to determine if ARID3a expression alters gene expression profiles in those cells that express ARID3a versus those that do not.

Increased plasma IL-10 levels, such as those the inventor observed in ARID3a<sup>H</sup> patient samples (FIG. 5), have been associated with inflammatory responses. The inventor observed increased numbers of ARID3a<sup>+</sup> B cells in two controls who were recovering from recent viral infections in the inventor's longitudinal studies. This suggested that ARID3a-expressing cells may be recruited as part of normal, and/or inflammatory, immune response. Although some ARID3a<sup>+</sup> cells clearly expressed the activation marker CD86, ARID3a expression did not segregate with expression of this marker (FIGS. 4A-B), an activation marker previously shown to be increased in SLE B lymphocytes (22). Nor did the inventor observe increased numbers of ARID3a<sup>+</sup> cells in any of six RA patient samples with similar levels of expression of the activation marker CD86. Therefore, ARID3a expression in SLE B cells did not always coincide with activation.

Expression of ARID3a in SLE naive B lymphocytes that only rarely show detectable ARID3a expression in healthy controls was unexpected. One explanation for the presence of both ARID3a<sup>+</sup> and ARID3a<sup>-</sup> naive B cells in SLE samples is that the ARID3a<sup>-</sup> fraction represents abnormal expansion of B lineage cells that constitutively express it. B 1 cells in the mouse may constitute a distinct B cell lineage (29). Mouse B1 B cells constitutively express Bright/ARID3a (21), and these cells were missing in Bright knockout mice (30). Preliminary data recently suggested CD43 was a marker for mature human B1 cells (31); however, others suggested these cells represent an intermediary pre-plasmablast phenotype (24). Regardless, ARID3a expression did not segregate with CD43 expression, so it is not currently possible to distinguish if ARID3a is a marker for a previously undescribed B cell subset.

Alternatively, ARID3a expression in SLE B cells may be a consequence, or a contributing factor of SLE. Although >40% of SLE patient samples showed large numbers of ARID3a<sup>+</sup> B cells, and increased numbers of these cells were associated with disease activity, RA blood samples did not exhibit expansion of ARID3a<sup>+</sup> cells. However, the inventor cannot rule out the possibility that examination of larger numbers of RA samples might show expansion of ARID3a<sup>+</sup> cells. Lupus is a polygenic disease, which is thought to arise due to imbalances at multiple regulatory levels of tolerance or immune activity. Immune pathology in lupus is known to be heterogeneous and there are many overlaps with other autoimmune diseases, therefore it is possible that aberrant ARID3a expression is not limited to SLE, or that it might occur transiently in certain healthy immune inflammatory responses. Likewise, ARID3a/Bright over-expression in mouse B lymphocytes caused ANA production, but was
insufficient to result in organ-threatening autoimmune pathology or early mortality (10,11).
Therefore, dysregulated ARID3a expression could be a predisposing or contributing factor to
disease requiring a permissive autoimmune environmental insult or additional genetic
predisposition to allow for clinical end-organ impact. Experiments to better define the
functions of ARID3a in SLE B cells may shed light on these questions.

Although tremendous progress has been made in identifying genetic associations with
SLE, biomarkers that have predictive potential for this diverse disease are essential (7,32,33).
Although ARID3a expression in several mature B cell subsets was strongly associated with
increased disease activity (Table 4), many patients also had dramatically increased numbers
of ARID3a+ naive B cells that are likely precursors to those mature cells. The inventor cannot
conclude from the study that numbers of ARID3a+ naive B cells might be predictive of future
disease flares, but it will be important to further explore that idea. The current results
showing strong associations with increased numbers of ARID3a+ B cells and SLEDAI scores
suggest ARID3a may either be useful as a novel biomarker for disease activity or perhaps
eventually be targeted for preventative treatment.

* * * * * * *

All of the compositions and methods disclosed and claimed herein can be made and
executed without undue experimentation in light of the present disclosure. While the
compositions and methods of this invention have been described in terms of preferred
embodiments, it will be apparent to those of skill in the art that variations may be applied to
the compositions and/or methods and in the steps or in the sequence of steps of the method
described herein without departing from the concept, spirit and scope of the invention. More
specifically, it will be apparent that certain agents that are both chemically and
physiologically related may be substituted for the agents described herein while the same or
similar results would be achieved. All such similar substitutes and modifications apparent to
those skilled in the art are deemed to be within the spirit, scope and concept of the invention
as defined by the appended claims.
X. References

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.


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CLAIMS

1. A method of predicting an auto-antibody flare in a subject with systemic lupus erythematosus (SLE) comprising:
   (a) obtaining a whole blood sample from a subject;
   (b) assessing the level of Bright/ARID3a in B-cells of said sample; and
   (c) predicting an auto-antibody flare when an elevated level of Bright/ARID3a in said B-cells, as compared to control B-cells from a subject not undergoing a disease flare, is observed.

2. The method of claim 1, wherein said B-cells are CD19+ B-cells.

3. The method of claim 1, wherein said control B-cells are from a health subject.

4. The method of claim 1, wherein said control B-cells are from a non-flaring SLE subject.

5. The method of claim 1, wherein assessing comprises immunologic detection of Bright/ARID3a.

6. The method of claim 5 wherein immunologic detection comprises flow cytometry, ELISA, RIA or Western blot.

7. The method of claim 1, wherein assessing comprises detection of a Bright/ARID3a transcript.

8. The method of claim 7, wherein detection of a Bright/ARID3a transcript comprises amplification of Bright/ARID3a mRNA.

9. The method of claim 8, wherein said amplification comprises RT-PCR.

10. The method of claim 1, further comprising performing a SLEDA Index analysis on said subject.

11. The method of claim 1, further assessing anti-nuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA) in a sample from said subject.
12. The method of claim 1, further comprising taking a medical history of said subject.

13. A method of determining the severity of systemic lupus erythematosus (SLE) in a subject:

(a) obtaining a whole blood sample from a subject;
(b) assessing the level of Bright/ARID3a in B-cells of said sample; and
(c) determining severe SLE when an elevated level of Bright/ARID3a in said B-cells, as compared to B-cells from a subject having moderate SLE, is observed.

14. The method of claim 13, wherein said B-cells are CD19+ B-cells.

15. The method of claim 13, wherein assessing comprises immunologic detection of Bright/ARID3a.

16. The method of claim 15, wherein immunologic detection comprises flow cytometry, ELISA, RIA or Western blot.

17. The method of claim 13, wherein assessing comprises detection of a Bright/ARID3a transcript.

18. The method of claim 17, wherein detection of a Bright/ARID3a transcript comprises amplification of Bright/ARID3a mRNA.

19. The method of claim 18, wherein said amplification comprises RT-PCR.

20. The method of claim 13, further comprising performing a SLEDA Index analysis on said subject.

21. The method of claim 13, further comprising assessing anti-nuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA) in a sample from said subject.

22. The method of claim 13, further comprising taking a medical history of said subject.

23. A method of predicting the progression of systemic lupus erythematosus (SLE) in a subject comprising:

(a) obtaining a whole blood sample from a subject;
(b) assessing the level of Bright/ARID3a in B-cells of said first sample;

c) obtaining a second B-cell containing sample from a subject;

d) assessing the level of Bright/ARID3a in B-cells of said second sample; and

e) predicting progression of SLE when an elevated level of Bright/ARID3a in B-cells of said second sample, as compared to B-cells from said first sample, is observed.

24. The method of claim 23, wherein said B-cells are CD19+ B-cells.

25. The method of claim 23, wherein assessing comprises immunologic detection of Bright/ARID3a.

26. The method of claim 25, wherein immunologic detection comprises flow cytometry, ELISA, RIA or Western blot.

27. The method of claim 23, wherein assessing comprises detection of a Bright/ARID3a transcript.

28. The method of claim 27, wherein detection of a Bright/ARID3a transcript comprises amplification of Bright/ARID3a mRNA.

29. The method of claim 28, wherein said amplification comprises RT-PCR.

30. The method of claim 23, further comprising performing a SLEDAI Index analysis on said subject.

31. The method of claim 21, further comprising assessing anti-nuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA) in a sample from said subject.

32. The method of claim 21, further comprising taking a medical history of said subject.

33. A method of assessing the efficacy of a treatment for systemic lupus erythematosus (SLE) in a subject comprising:

(a) obtaining a first whole blood sample from a subject;

(b) assessing the level of Bright/ARID3a in B-cells of said first sample;
(c) treating said subject for with an SLE treatment;

(d) obtaining a second whole blood sample from a subject; and

(e) assessing the level of Bright/ARID3a in B-cells of said second sample,

wherein a reduced level of Bright/ARID3a in B-cells of said second sample, as compared to B-cells from said first sample, indicates that said SLE treatment was effective.

34. The method of claim 33, wherein said B-cells are CD19+ B-cells.

35. The method of claim 33, wherein assessing comprises immunologic detection of Bright/ARID3a.

36. The method of claim 35, wherein immunologic detection comprises flow cytometry, ELISA, RIA or Western blot.

37. The method of claim 33, wherein assessing comprises detection of a Bright/ARID3a transcript.

38. The method of claim 37, wherein detection of a Bright/ARID3a transcript comprises amplification of Bright/ARID3a mRNA.

39. The method of claim 38, wherein said amplification comprises RT-PCR.

40. The method of claim 33, further comprising performing a SLEDA Index analysis on said subject.

41. The method of claim 33, further comprising assessing anti-nuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA) in a sample from said subject.

42. The method of claim 33, further comprising taking a medical history of said subject.

43. A method of treating a subject with systemic lupus erythematosus (SLE) in a subject:

(a) obtaining a whole blood sample from a subject;

(b) assessing the level of Bright/ARID3a in B-cells of said sample; and
(c) treating said subject for SLE when said B-cells exhibit an elevated level of Bright/ARID3a as compared to B-cells from a healthy control.

44. The method of claim 43, wherein said B-cells are CD19+ B-cells.

45. The method of claim 43, wherein assessing comprises immunologic detection of Bright/ARID3a.

46. The method of claim 45, wherein immunologic detection comprises flow cytometry, ELISA, RIA or Western blot.

47. The method of claim 43, wherein assessing comprises detection of a Bright/ARID3a transcript.

48. The method of claim 47, wherein detection of a Bright/ARID3a transcript comprises amplification of Bright/ARID3a mRNA.

49. The method of claim 48, wherein said amplification comprises RT-PCR.

50. The method of claim 43, further comprising performing a SLEDA Index analysis on said subject.

51. The method of claim 43, further comprising assessing anti-nuclear antibody (ANA) testing and anti-extractable nuclear antigen (anti-ENA) in a sample from said subject.

52. The method of claim 43, further comprising taking a medical history of said subject.

53. A kit comprising:

   (a) one or more reagents for assessing the expression of Bright/ARID3a in a biological sample; and

   (b) one or more reagents for assessing anti-nuclear antibody (ANA) testing and/or anti-extractable nuclear antigen (anti-ENA) in a biological sample.

54. The kit of claim 53, further comprising an agent for treating systemic lupus erythematosus.

55. The kit of claim 54, wherein said agent is a Bright/ARID3a inhibitor.
56. The kit of claim 55, wherein said Bright/ARID3a inhibitor is selected from the group consisting of an interfering RNA, a dominant-negative Bright/ARID3a molecule, a Bright/ARID3a peptide or an expression vector coding therefor.

57. The kit of claim 54, wherein said agent is a corticosteroid or an anti-malarial drug.
Fig. 1B

ARID3a

β-actin

Fig. 1C

ARID3a+CD19+ Cells/ml

SLE  Ctrl  RA

Fig. 1D

SLEDAI

ARID3a^H  ARID3a^N

0.017
FIG. 7 (Cont'd.)

- Memory Switched
- Plasma Cells
- Plasmablasts
- Naïve IgD
Spearman $r=0.298$, $P=0.006$

**ARID3a CD19**
(adjusted for the total B cell counts)

**FIG. 9**
### INTERNATIONAL SEARCH REPORT

**International application No.**  
PCT/US2014/036881

### A. CLASSIFICATION OF SUBJECT MATTER

**IPC(8) -** G01N 33/50 (2014.01)
**CPC -** C07K 14/4713

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

**Minimum documentation searched (classification system followed by classification symbols):**
- IPC(8) - A61K 38/17; A61P 19/02; G01N 33/50 (2014.01)
- USPC - 424/131.1; 514/12.2, 16.6;

**Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched**
- CPC - A61K 9/0019; 38/1709; C07K 14/4713 (2014.09)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
- PatBase, Google Patents, Google Scholar, PubMed

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td><strong>SHANKAR et al.</strong> &quot;Anti-Nuclear Antibody Production and Autoimmunity in Transgenic Mice That Overexpress the Transcription Factor Bright,&quot; The Journal of Immunology, 01 March 2007 (01.03.2007), Vol. 178, Pgs. 2996-3006. entire document</td>
<td>1-3, 5-9</td>
</tr>
<tr>
<td>Y</td>
<td><strong>US 2012/0225790</strong> A1 (JULIA CANO et al) 06 September 2012 (06.09.2012) entire document</td>
<td>33-42</td>
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</table>

Further documents are listed in the continuation of Box C.

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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11 September 2014

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