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(54) Title: USE OF STATIN TO KILL EBV-TRANSFORMED B CELLS

(57) Abstract: Simvastatin, other LFA-1 inhibiting statins, and LFA-1 inhibiting statin-derived and statin-like compounds, are useful for treatment or prevention of EBV-associated (or herpes virus-associated or other virus-associated) tumors, including lymphomas and carcinomas, expressing LFA-1 and transforming proteins.

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USE OF A STATIN TO KILL EBV-TRANSFORMED B CELLS

Related Applications

This application claims the benefit of the US Provisional Application No.: 60/515,013 filed October 28, 2003, the disclosure of which is hereby incorporated by reference in its entirety.

Field of the Invention

Simvastatin, other LFA-1 inhibiting statins, and LFA-1 inhibiting statin-derived and statin-like compounds, are useful for treatment or prevention of EBV-associated (or herpes virus-associated or other virus-associated) tumors, including lymphomas and carcinomas, expressing LFA-1 and transforming proteins.

Background of the Invention

Infection of primary B cells with Epstein-Barr virus (EBV) results in transformation with growth of the cells in tight clumps and immortalization of the cells. These immortalized B cells have an immunoblastic morphology, and express each of the EBV nuclear antigens (EBNAs) and latent membrane proteins (LMPs) (Kieff, E. & Rickinson, A. 2001 in: Fields Virology, eds. Knipe, D. M. & Howley, P. M. Lippincott Williams & Wilkins, Philadelphia, Vol. 2, pp. 2511-2573; Rowe, M. et al. 1992 *J Virol* **66**:122-31). EBNA-2 is a transactivator that up-regulates expression of cellular genes and LMPs. LMP-1 is an oncoprotein that constitutively activates nuclear factor κB (NF-κB) to induce B cell proliferation (Mosialos, G. et al. 1995 *Cell* **80**:389-99). LMP-1 also induces expression of adhesion molecules leukocyte function antigen-1 (LFA-1), LFA-3, and intercellular adhesion molecule-1 (ICAM-1) on the surface of EBV-transformed B cells (Peng, M. & Lundgren, E. 1992 *Oncogene* **7**:1775-82; Wang, D. et al. 1988 *J Virol* **62**:4173-84). The high level expression of adhesion molecules contributes to the clumping of EBV-infected B cells *in vitro* (Rothlein, R. & Springer, T.A. 1986 *J Exp Med* **163**:1132-1149).

EBV-associated immunoblastic lymphomas occur in immunocompromised patients such as those with AIDS or transplant recipients (Cohen, J.I. 2000 *N Engl J Med* **343**:481-92; Carbone, A. 2003 *Lancet Oncol* **4**:22-9). Because these EBV-associated immunoblastic lymphomas express each of the EBNAs and LMPs (Carbone, A. 2003 *Lancet Oncol* **4**:22-9) that induce proliferation of B cells, the virus is thought to be directly responsible for the pathogenesis of these tumors (Mosialos, G. et al. 1995 *Cell* **80**:389-99). LMP-1 in EBV-

associated lymphoma cells binds to tumor necrosis factor receptor associated factors and the tumors show activation of NF-κB (Liebowitz, D. 1998 *N Engl J Med* **338**:1413-21). Many immunocompromised patients with EBV-associated immunoblastic lymphoma have tumors at extranodal sites such as the brain, lung, or gastrointestinal tract. The high-level expression of LFA-1 and other cellular adhesion molecules in these tumors may contribute to their extranodal location (Hamilton-Dutoit, S. J. et al. 1993 *Am J Pathol* **143**:1072-85). The prognosis of these lymphomas is often poor for patients with irreversible immunosuppression and treatment options are limited.

Simvastatin is a member of the statin family of drugs that inhibit 3-hydroxy-3-methylglutaryl CoA reductase (Lennernas, H. & Fager, G. 1997 *Clin Pharmacokinet* **32**:403-25). Statins lower plasma cholesterol levels, resulting in reduction of the risk of cardiovascular disease (Corsini, A. et al. 1995 *Pharmacol Res* **31**:9-27). Weitz-Schmidt *et al.* demonstrated that certain statins including simvastatin and lovastatin, bind to the I (inserted) domain of LFA-1 and inhibit its function (Weitz-Schmidt, G. et al. 2001 *Nat Med* 7:687-92). In contrast, other statins such as pravastatin do not bind to LFA-1 (Weitz-Schmidt, G. et al. 2001 *Nat Med* 7:687-92). LFA-1 is expressed on the surface of various leukocytes and plays an important role in cell adhesion and costimulation of T cells. The I domain of LFA-1 is the binding site for ICAM-1, a ligand of LFA-1 (Randi, A. M. & Hogg, N. 1994 *J Biol Chem* **269**:12395-8.; Knorr, R. & Dustin, M. L. 1997 *J Exp Med* **186**:719-30). The binding of simvastatin or lovastatin to the LFA-1 I domain induces a conformational change in LFA-1, resulting in inhibition of the interaction of LFA-1 with ICAM-1 (Weitz-Schmidt, G. et al. 2001 *Nat Med* 7:687-92). As a result of their binding to LFA-1, these statins inhibit the costimulatory activity of LFA-1 and suppress the inflammatory response in a murine model of peritonitis (Weitz-Schmidt, G. et al. 2001 *Nat Med* 7:687-92).

Segue to the Description

Here, we investigate the ability of simvastatin and other statins to inhibit EBV-positive B cell proliferation. Because simvastatin binds to and inhibits the function of LFA-1, we postulated that the drug would inhibit the growth of these cells both *in vitro* and *in vivo*. Inoculation of EBV-transformed lymphoblastoid cell lines (LCLs) into severe combined immunodeficiency (SCID) mice results in the formation of EBV-associated immunoblastic lymphomas that contain EBV genomes and express EBNAs, LMPs, and adhesion molecules including LFA-1 (Rowe, M. et al. 1991 *J Exp Med* **173**:147-58.;

Picchio, G. R. et al. 1993 *Am J Pathol* **143**:342-9). In the present study, we treated EBV-transformed LCLs *in vitro* with simvastatin, and administered the drug and inoculated SCID mice with LCLs to assess development of B cell lymphomas.

Summary of the Invention

Simvastatin, other LFA-1 inhibiting statins, and LFA-1 inhibiting statin-derived and statin-like compounds, are useful for treatment or prevention of EBV-associated (or herpes virus-associated or other virus-associated) tumors, including lymphomas and carcinomas, expressing LFA-1 and transforming proteins.

Brief Description of the Drawings

Figure 1. Structures of statins and the statin-derived lymphocyte-function-associated antigen 1 (LFA-1) inhibitor LFA703 (Weitz-Schmidt, G. 2002 *Trends Pharmacol Sci* **23:**482-86).

Figure 2. Schematic representation of the mevalonate pathway (Weitz-Schmidt, G. 2002 *Trends Pharmacol Sci* **23:**482-86).

Figure 3. Interaction between lymphocyte-function-associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1) (Weitz-Schmidt, G. 2002 *Trends Pharmacol Sci* **23:** 482-86).

Figure 4. Chemical structures of small molecule integrin inhibitors (Shimaoka, M. *et al.* 2003 *Immunity* **19:** 391-402) **A.** α/β I-like allosteric inhibitors are shown: Compounds 1, 3, and 4 (Gadek, T.R. *et al.* 2002 *Science* **295:**1086-89), and Compound 5 (Fotouhi, N. *et al.* 20 Apr. 2000 WO 00/21920) (Welzenbach, K. *et al.* 2002 *J Biol Chem* **277:**10590-98). **B.** α1 allosteric inhibitors are shown: BIRT377 (Last-Barney, K. *et al.* 2001 *J Am Chem Soc* **123:**5643-50), LFA703 (Weitz-Schmidt, G. *et al.* 2001 *Nat Med* **7:**687-92), and A-286982 (Liu, G. *et al.* 2001 *J Med Chem* **44:** 1202-10).

Figure 5. Simvastatin inhibits clump formation, and induces apoptosis in LCLs. (**A**). Cell clumping of LCLs was observed after simvastatin was added at 0 - 2 μM for 5 days. (**B**). XTT ell proliferation assay was performed after addition of simvastatin (0 - 8 μM) to various cells lines for 7 days. Error bars indicate standard deviations for four independent experiments. (**C**). Cell viability was assayed by trypan blue staining after cell lines were cultured with 0 - 8 μM simvastatin for 7 days. Error bars indicate standard deviations for three separate experiments. (**D**). Percentage of dead cells in the absence (open bars) or presence (filled bars) of 2 μM simvastatin for 7 days as determined by PI staining and flow cytometry. PI-positive cells were counted as dead cells. (**E**). PI staining.

Cells were treated with or without simvastatin for 5 days. Cell populations in sub-G0-G1 and G0-G1-S-M phase are indicated. **(F).** TUNEL assays were performed for cells treated with 2 μM simvastatin for 5 days (gray area with solid line), no simvastatin for 5 days (white area with solid line) or serum starvation for 72 hr (white area with dotted line). **(G).** DNA ladder formation for cells cultured with (+) or without (-) 2 μM of simvastatin for 5 days.

Figure 6. Apoptosis-inducing effect of simvastatin depends on binding to the I domain of LFA-1. **(A)** and **(B).** XTT cell proliferation assay was performed for LCLs 12A1 (**A**) and 6B10 (**B**) in the presence of simvastatin or pravastatin after 5 days. **(C).** Cell viability was assayed by trypan blue staining for LCL 12A1 in the presence of anti-LFA-1 antibody TS1/22, which recognizes the I domain of LFA-1, or an isotype control antibody for 7 days. **(D).** Cell viability was measured by trypan blue staining of LCLs 12A1 and 295H cultured with soluble ICAM-1 (sICAM-1) for 7 days. **(E).** LCL 12A1 was cultured with anti-LFA-1 (TS1/22) or isotype control antibody for 1 h, simvastatin (2 μM) or no compound was added, and the cells were cultured for 7 days. Cell clumping is reduced with isotype control antibody and simvastatin (upper), but not with anti-LFA-1 antibody and simvastatin (lower). **(F).** Cell viability was assayed by trypan blue staining of LCLs cultured with an anti-LFA-1 or isotype control antibody. Error bars indicate standard deviations of three separate experiments.

Figure 7. Simvastatin alters the localization of LMP-1, displaces LMP-1 from lipid rafts, and inhibits NF-κB activation in LCLs. **(A).** Immunofluorescence of LMP-1 in LCL 6B10 in the absence or presence of simvastatin or pravastatin. Cells were fixed, permeabilized, and incubated with anti-LMP-1 antibody (CS1-4, Dako Cytomation, Carpinteria, CA) followed by FITC-conjugated secondary antibody and PI. LMP-1 is white (green) (FITC) and nuclei are gray (red) (PI). **(B).** Immunoblotting of LCL fractions. LCLs were cultured in the absence or presence of simvastatin or pravastatin for 3 days and cell lysates were fractionated by sucrose gradient ultracentrifugation. Equal aliquots of each fraction beginning at the top of the centrifuge tube were probed with antibodies to LMP-1, Lyn (a tyrosine kinase that localizes in lipid rafts), and CD71 (a transferrin receptor that does not localize in rafts). **(C).** Electrophoretic mobility shift assays for NF-κB. LCL 6B10 was cultured in the absence or presence of simvastatin or pravastatin for 3 days, and nuclear extracts were used in electrophoretic mobility shift assays with a radiolabeled NF-κB probe. Specific or non-specific non-radioactive competitor oligonucleotides were added

to some assays. The intensity of NF-κB-specific bands was measured by using a phosphorimager.

Figure 8. Simvastatin prolongs survival of SCID mice inoculated with LCLs. **(A).** Appearance of mice at 45 days after inoculation. The mouse that did not receive simvastatin (upper left, lower mouse) has ascites, but the simvastatin-treated mouse (upper mouse) has no ascites. The simvastatin-treated mouse (upper right) has no ascites or solid tumors, whereas the untreated mouse (lower right) has ascites (arrows) and solid tumors (arrowheads). Immunoblastic lymphoma is present in the mouse not treated with simvastatin (lower left). **(B).** Kaplan-Meier survival curves of SCID mice that were inoculated with 1.0×10^6 6B10 LCLs. Mice were pre-treated with simvastatin beginning 3 days before inoculation with cells (blue circles), treated with drug beginning 7 days after inoculation with cells (triangles), or not treated with drug (squares).

Figure 9. A left. Cell surface expression of LFA-1 in various cell lines. Gray peaks indicate expression of LFA-1 using anti-LFA-1 antibody G25.2, as the primary antibody. White peaks indicate isotype controls. Similar results were obtained using anti-LFA-1 antibody TS1/22 (1:100 dilution). **A right.** Expression of ICAM-1 in various cell lines. Cells were incubated with phycoerythrin-conjugated anti-ICAM-1 antibody (HA58, BD Pharmingen). Intensity of PE was measured by FACS (gray peaks). White peaks indicate isotype controls. **B left.** Expression of LMP-1 in various cell lines. Cells were lysed in sample buffer and immunoblotted with anti-LMP-1 (S12, BD Pharmingen). Lysates corresponding to 2×10^5 cells were loaded in each lane. **B right.** Activation of NF-κB in various cell lines. One μg of nuclear extracts were tested in electrophoretic mobility shift assays with a radiolabeled NF-κB probe.

Figure 10. **(A)** Lovastatin, **(B)** atorvastatin, and **(C)** simvastatin kill EBV-transformed cells. Percentage of dead cells in the absence (open bars) or presence (filled bars) of statin for 7 days as determined by PI staining and FACS. PI-positive cells were counted as dead cells.

Detailed Description of the Preferred Embodiment

The preferred embodiment provides use of simvastatin, other LFA-1 inhibiting statins, and LFA-1 inhibiting statin-derived and statin-like compounds, for treatment or prevention of an EBV-associated (or herpes virus-associated or other virus-associated) tumor, including lymphoma and carcinoma, expressing LFA-1 and transforming proteins. One embodiment provides use of an effective amount of an inhibitor of 3-hydroxy-3-

methylglutaryl coenzyme A (HMG CoA) reductase or homologues of the inhibitor. Cancers associated with EBV include nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's disease, lymphoproliferative disease, T-cell lymphoma, and non-Hodgkin's lymphoma (Cohen, J .I. 2000 *N Engl J Med* **343**:481-92). EBV is a member of the herpes virus family. Nasopharyngeal carcinomas express EBV proteins, such as LMP-1. In Burkitt's lymphoma, about 50% of lymphomas are associated with EBV, although they do not express LMP-1. In Hodgkin's disease, about 50% of lymphomas are associated with EBV, and they express EBV proteins, such as LMP-1. In lymphoproliferative disease, patients with immunodeficiencies have impaired T-cell immunity and are unable to control the proliferation of EBV-infected B cells so that their tissues show lymphomas, which express EBV proteins, such as LMP-1. T-cell lymphomas express EBV proteins, such as LMP-1. In non-Hodgkin's lymphoma in patients with AIDS, about 50% of lymphomas are associated with EBV. LMP-1 acts as an oncogene and up-regulates the expression of LFA-1.

"Homologue(s)" refers to compounds of similar chemical structure but which have different radicals or side groups substituted. The term is further meant to include analogues or compounds with similar electronic structures but different atoms; isolog is a synonym.

The method comprises treatment or prevention of a neoplasm. Administration of the compound is by any medically or pharmaceutically accepted route. Typically, the oral route is preferred. The dosing range is preferably from about 0.6 to about 148.5 mg/kg/day based on the patient's body weight. More specifically, the range is from about 2.0 to about 45.0 mg/kg/day.

Examples of compounds useful in the present invention are lovastatin, atorvastatin, and simvastatin, as well as their homologues. Also included are compounds classified as HMG Co-A reductase inhibitors, as well as their homologues. Generally, these HMG Co-A reductase inhibitors are known to lower serum cholesterol in humans. However, the present invention is not so limited. That is, an inhibitor of HMG Co-A reductase or one of its homologues may work in the method of the present invention without necessarily lowering serum cholesterol. The invention focuses not on the compound's ability to lower cholesterol, but rather on the compound's ability to treat selected cancers, such as EBV-associated (or herpes virus-associated or other virus-associated) tumors, including lymphomas and carcinomas, expressing LFA-1 and transforming proteins.

Another embodiment provides use of simvastatin, other LFA-1 inhibiting statins, and LFA-1 inhibiting statin-derived and statin-like compounds, for reduction of levels of an identifiable tumor marker in a patient having an EBV-associated (or herpes virus-associated or other virus-associated) tumor, including lymphoma and carcinoma, expressing LFA-1 and transforming proteins. Still another embodiment provides use of simvastatin, other LFA-1 inhibiting statins, and LFA-1 inhibiting statin-derived and statin-like compounds, for treatment or prevention of an EBV-associated (or herpes virus-associated or other virus-associated) tumor, including lymphoma and carcinoma, expressing LFA-1 and transforming proteins in conjunction with another treatment modality. Additionally described is an appropriate unit dose ranging from about 40 mg to about 3150 mg.

Simvastatin, other LFA-1 inhibiting statins, and LFA-1 inhibiting statin-derived and statin-like compounds

Statins represent a well-established class of drugs that effectively lower serum cholesterol levels and are widely prescribed for the treatment of hypercholesterolemia (Weitz-Schmidt, G. 2002 *Trends Pharmacol Sci* **23**:482-86). They can be grouped into natural compounds such as lovastatin, simvastatin, pravastatin and mevastatin, and fully synthetic compounds such as fluvastatin and atorvastatin, which are marketed, and rosuvastatin and pitavastatin, which are in late-stage clinical development (Fig. 1). Statins competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the enzyme that catalyzes the rate-limiting step of the cholesterol synthesis pathway in the liver and other tissues (Fig. 2). By inhibiting HMG CoA reductase, statins reduce cholesterol levels and might also lower intracellular levels of isoprenoids, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate (Fig. 2).

Fig. 1 illustrates structures of statins and the statin-derived lymphocyte-function-associated antigen 1 (LFA-1) inhibitor LFA703. (a) Lovastatin, simvastatin, pravastatin and mevastatin are natural statins. (b) Fluvastatin, atorvastatin, rosuvastatin and pitavastatin are fully synthetic statins. (c) LFA703 is a statin-derived compound designed to potently inhibit the β2 integrin LFA-1. The IC_{50} (median inhibitory concentration) and Ki (inhibition constant) values for 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibition range from 0.1 to 28 nm. By contrast, LFA703 does not inhibit HMG CoA reductase up to a concentration of 100 μm.

Fig. 2 illustrates a schematic representation of the mevalonate pathway. Statins block the conversion of 3-hydroxy-3-methylglutaryl-CoA to mevalonate. This leads to

reduced synthesis of cholesterol and decreased prenylation of proteins such as small guanosine triphosphate-binding proteins. The dashed arrow indicates that there are several other biosynthetic steps necessary to convert mevalonate into farnesyl pyrophosphate.

The common structural characteristic of all statins is a side chain that exists either in a closed ring (inactive, lactone) or an open ring (active, acid) form (Wong, WW-L. *et al.* 2002 *Leukemia* **16:**508-19). The former undergoes activation in vivo by carboxyesterases in plasma and liver. The open ring conformation of this drug blocks catalytically active HMG CoA reductase by functioning as a molecular mimic of a reaction intermediate formed within the active site of this enzyme. Statins are effective competitive inhibitors as they bind HMG CoA reductase with high affinity.

Each member of the statin family of drugs functions by a similar mechanism of action but maintains unique binding affinities, pharmacokinetics and dosing levels (Table 1). In brief, the binding affinities of the inhibitors (Ki) range from 0.1 to 2.3 nM, whereas the K_m of the natural substrate, HMG CoA is 4 μM. The pharmacokinetics are disparate and largely dictated by their lipophilic nature, acid or lactone form, and mechanism of cytochrome P450 metabolism in the liver, which is the primary site of action for cholesterol control. Briefly, statin action for hypercholesterolemia leads to a decrease in intracellular hepatic cholesterol levels which then induces expression of cell surface low-density lipoprotein receptors, enabling cholesterol to be removed from the circulation and replenish intracellular cholesterol stores. The safety of this family of drugs has been documented extensively and they are remarkably well tolerated. Reports of minor adverse side-effects include constipation, flatulence, dyspepsia, nausea, gastrointestinal pain, and elevated serum transaminase levels. The most serious adverse effect is myotoxicity including rhabdomyolysis which can be diminished with co-administration of ubiquinone.

Table 1. Characteristics of Statins

Statin	Lipo-philic	Form	Source	Ki for HMG CoA reductase (nM)	IC50 for HMG CoA reductase activity (nM) in rat liver microsomes	Major metabolism by P450	Dosage for chole-sterol reduction (mg/day)
Lovastatin	yes	lactone	fungi	0.6	20	3A4	20-80
Simvastatin	yes	lactone	fungi	0.12	18.1	3A4	10-80
Pravastatin	no	acid	fungi	2.3	55.1	minimal	10-40
Fluvastatin	yes	acid	synthetic	0.3	17.9	2C9, 2D6	20-80
Atorvastatin	yes	acid	synthetic	NA	15.2	3A4	10-80
Cerivastatin	yes	acid	synthetic	1.3	13.1	3A4, 2C8	0.2-0.4
Rosuvastatin	no	acid	synthetic	0.1	11.8	2C9, 2C19	5-80
Pitavastatin	yes	acid	synthetic	1.7	6.8	2C9, 2C18	4

High-throughput screening of large chemical libraries has identified lovastatin as an extracellular inhibitor of LFA-1 (Weitz-Schmidt, G. 2002 *Trends Pharmacol Sci* **23**:482-86). Lovastatin was shown to decrease LFA-1-mediated leukocyte adhesion to ICAM-1 and T-cell co-stimulation. The inhibition of LFA-1 by lovastatin is highly specific because other integrins, including Mac-1 and VLA-4, were unaffected. Unexpectedly, lovastatin was found to bind to a hitherto unknown site in the LFA-1 I (inserted) domain, as documented by nuclear magnetic resonance spectroscopy and crystallography (Fig. 3). This site, termed the 'lovastatin site' (L-site), is distant from the ICAM-1-binding site, which indicates that lovastatin inhibits LFA-1 via an allosteric mechanism (Fig. 3). In agreement with this hypothesis, LFA-1 containing a mutant I domain that is locked in the active, ligand-binding conformation is completely resistant to inhibition by lovastatin. Thus, there is strong evidence that lovastatin inhibits LFA-1 function by stabilizing the receptor in an inactive conformation. In addition to lovastatin, simvastatin and mevastatin also inhibit LFA-1 by binding to the L-site.

Fig. 3 illustrates the interaction between lymphocyte-function-associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1). (a) As established for other integrins, the β2 integrin LFA-1 requires activation by intracellular signals before binding to its major ligand ICAM-1. This activation converts LFA-1 from an inactive to an active state by inducing conformational changes in the extracellular domains of LFA-1. Several statins bind to a regulatory site within the so-called inserted (I) domain of LFA-1, thereby locking LFA-1 in an inactive state. (b) The crystal structure of the complex between the

LFA-1 I domain and lovastatin shows that the regulatory "lovastatin site" (L-site) is located distant from the metal-ion-dependent adhesion site (MIDAS). The MIDAS is a divalent cation coordination site within the I domain that is important for ICAM-1 binding.

Guided by the crystal structure of the complex between the LFA-1 I domain and lovastatin (Fig. 3), a series of statin-like compounds, including the LFA-1 inhibitor LFA703, has been synthesized (Fig. 1). These compounds strongly inhibit LFA-1 but do not affect the activity of HMG CoA reductase. LFA703 binds to the L-site on the I domain of LFA-1 and is more potent than lovastatin in LFA-1 binding assays.

Another statin-like compound, the LFA-1 inhibitor LFA451, has been synthesized (Welzenbach, K. *et al.* 2002 *J Biol Chem* **277:**10590-98). LFA451 blocks LFA-1 and does not inhibit the activity of HMG CoA reductase. The molecular target for LFA-1 inhibition by LFA451A is the I-like domain that is different from the regulatory L-site on the I domain of LFA-1, demonstrating that other domains constitute suitable targets for the design of other statin-like LFA-1 inhibitors.

At least two distinct classes of potent, low molecular weight αLβ2 antagonists are under development that have different binding sites. LFA-1 (αLβ2) is a member of the leukocyte integrin subfamily (Shimaoka, M. *et al.* 2003 *Immunity* **19:**391-402). αLβ2 plays a critical role in leukocyte adhesion to and migration through endothelium by its ability to bind intercellular adhesion molecules (ICAMs), especially ICAM-1, on endothelial cells. Leukocyte integrins contain one von Willebrand factor-type A domain in each subunit, the inserted (I) domain in the α subunit and the I-like domain in the β subunit. Each domain adopts an α/β Rossmann fold with a metal ion-dependent adhesion site (MIDAS) on the "top" of the domain, whereas its C- and N-terminal connections to the neighboring domain are on the distal, "bottom" face (Fig. 3). αLβ2 binds its immunoglobulin superfamily ligand ICAM-1 through the αL I domain, with the binding site centered on the Mg^{2+} of the MIDAS which directly coordinates to ICAM-1. The affinity of the αL I domain for ICAMs is regulated by downward axial displacement of its C-terminal helix, which is conformationally linked to alterations of MIDAS loops and Mg^{2+} coordination. The β2 I-like domain does not directly bind ligand despite containing a MIDAS motif. Instead, it functions indirectly by regulating the activity of the I domain. One group of antagonists binds underneath the C-terminal α helix of the αL I domain, *e.g.,* LFA703 (Novartis), BIRT377 (Boehringer-Ingelheim), and A-286982 (ICOS/Abbott/Biogen), blocks the downward axial displacement of the C-terminal helix, and inhibits ligand binding of αLβ2

allosterically by stabilizing the I domain in the low-affinity conformation (Fig. 4). By contrast, a second class of compounds, which has been patented as αLβ2 antagonists by Genentech (*e.g.*, Compounds 1, 3, and 4) or by Roche (*e.g.*, Compound 5) has a different mechanism of action (Fig. 4). Shimaoka, M. *et al.* demonstrate that these inhibitors represent a second class of antagonists of I domain-containing integrins, which bind to the β2 I-like domain MIDAS near a key regulatory interface with the αL subunit, and block communication of conformational change to the I domain, while at the same time activating conformational rearrangements elsewhere in integrins. The results of the study by Shimaoka, M. *et al.* highlight another target for integrin antagonism by small molecules and other statin-derived and statin-like compounds, the use of which LFA-1 inhibiting statin-derived and statin-like compounds, besides LFA-1 inhibiting statins, is envisioned in the present invention.

Apoptosis

In recent years it has become clear that inhibitors of HMG CoA reductase can trigger a subset of tumor-derived cells to undergo apoptosis (Wong, WW-L. *et al.* 2002 *Leukemia* **16:**508-19). Tumor cell types that undergo apoptosis upon exposure to lovastatin include acute myelogenous leukemia (AML), juvenile monomyelocytic leukemia, rhabdomyosarcoma, squamous cell carcinoma of the cervix and of the head and neck; medulloblastoma, mesothelioma, pancreatic carcinoma, neuroblastoma and astrocytoma. For the first time we extend the list to EBV-transformed cells and herpes virus-infected or other virus-infected cells of hematopoietic origin.

Analyses of cell culture and animal models of carcinogenesis have shown that statins can decrease tumor cell number alone and in combination with other anti-cancer agents. When administered as the sole agent in animal models statins can decrease tumor load of AML, melanoma, hepatoma, pancreatic, lung and neuroblastoma. However, it is unlikely that an agent will be effective in eradicating disease when administered by itself, hence multiagent cancer therapy is practiced. In particular, lovastatin has been shown to potentiate antitumor activity of doxorubicin, TNF-α, carmustine (BCNU; N, N'-bis(2-chloroethyl)-N-nitrosourea) in mouse tumor models of lung, colon carcinoma, melanoma and astrocytoma. Other statins, including lovastatin have been shown to potentiate apoptotic effects of cytosine arabinoside, phenylacetate, cisplatin, 5-fluorouracil, butyrate and non-steroidal anti-inflammatory drugs (NSAIDs) such as sulindac in AML, glioblastoma, and colon cancer cells. Identifying agents that synergize with statins will aid

in their proper application in the clinic and may also help elucidate the mechanism of statin-induced apoptosis.

EBV-associated (or herpes virus-associated or other virus-associated) tumors

Membership in the herpes family is based on the architecture of the virion. A typical herpes virion consists of a core containing a linear double-stranded DNA, an icosahedral capsid approximately 100 to 110 nm in diameter containing 162 capsomeres with a hole running down the long axis, and an envelope containing viral glycoprotein spikes on its surface. Thus far, nine herpesviruses have been isolated from humans: herpes simplex virus 1, herpes simplex virus 2, human cytomegalovirus, varicella-zoster virus, Epstein-Barr virus (EBV), and human herpesviruses 6A, 6B, 7, and 8 known as Kaposi's sarcoma-associated herpesvirus (KSHV).

EBV became the first candidate human tumor virus. EBV is now the prototype of the gamma subfamily of potentially oncogenic herpesviruses. The gamma herpesvirus subfamily includes both the gamma 1, or Lymphocryptovirus (LCV), and gamma 2, or Rhadinovirus (RDV), genera. EBV is the only known human LCV, and the recently discovered Kaposi's sarcoma-associated herpesvirus (KSHV) is the only known human RDV.

Overall, the LCV and RDV genomes are much more closely related to each other than to the genomes of the alpha or beta herpesviruses. Of the 75 KSHV predicted ORFs, 54 are colinearly homologous to ORFs of EBV. The mean amino acid identity to EBV among the 54 KSHV ORFs is 35%, with only two less than 21%.

The gamma herpesvirus subfamily classification was initially established not on the basis of similarity in genome organization but on the basis of similarity in biologic properties. EBV and KSHV have particularly limited host ranges for *in vitro* infection and are associated with human malignancies. Much of the interest in these viruses is because of their association with cancer.

EBV readily causes infection in human B lymphocytes. The usual outcome of infection with EBV is latency. EBV can also establish latent infection in other cell types, including T or natural killer (NK) cells.

The genes encoding the EBV latent infection nuclear and membrane proteins have been identified. Six EBV nuclear antigens (EBNAs) and two latent membrane proteins (LMPs) were found to be involved in latency and cell growth transformation. LMP-1 was

found to have significant transforming activity and to induce increased expression of LFA-1.

EBV is strongly linked to seven types of human malignancy. Table 2 summarizes the essential information regarding these seven tumor types, the length of the latency period between primary EBV infection (or initiation of immune suppression) and tumor development, the strength of the viral association, and the extent of viral antigen expression in tumor cells (Rickinson, A. & Kieff, E. 2001 in: Fields Virology, eds. Knipe, D.M. & Howley, P.M. Lippincott Williams & Wilkins, Philadelphia, Vol. 2, pp. 2575-2627).

Table 2. Overview of EBV-associated malignancies

Tumor	Subtype	Typical latent period[a]	EBV association (%)[b]	EBV antigen expression[c]	Latency
Burkitt's lymphoma	Endemic Sporadic AIDS-associated	3-8 y post-EBV 3-8 y post-EBV 3-8 y post-HIV	100 15-85 30-40	EBNA1	I
Gastric carcinoma	UCNT Adenocarcinoma	>30 y post-EBV >30 y post-EBV	100 5-15	EBNA1, LMP2	I/II
Nasopharyngeal carcinoma	Nonkeratinizing Keratinizing	>30 y post-EBV >30 y post-EBV	100 100	EBNA1, LMP1, LMP2	I/II
T-cell lymphoma	VAHS-associated Nasal NK and T-cell	1-2 y post-EBV >30 y post-EBV	100 100	EBNA1, LMP1, LMP2	I/II
Hodgkin's disease	Mixed cell, lymphocyte depleted Nodular sclerosing	>10 y post-EBV >10 y post-EBV	60-80 20-40	EBNA1, LMP1, LMP2	II
Lymphoproliferative disease-associated lymphoma	Immunodeficiency Posttransplantation AIDS-associated	<3 mo post-EBV <1 y posttransplantation >8 y post-HIV	100 >90 >80	EBNA 1, 2, 3A, 3B, 3C,-LP, LMP1, LMP2	III
Leiomyosarcoma	Immunodeficiency Posttransplantation AIDS-associated	?<3 y post-EBV ?<3 y posttransplantation ?<3 y post-EBV	?100 ?100 ?100	?	?

[a]Typical latent period between EBV infection and tumor development, or where appropriate, between onset of T-cell impairment (transplantation or HIV infection) and tumor development.

[b]Percentage of tumors that are EBV genome positive.

[c]Antigen expression is identified by monoclonal antibody staining or is inferred from analysis of latent gene transcripts.

AIDS, acquired immunodeficiency syndrome; UCNT, undifferentiated carcinomas of nasopharyngeal type; VAHS, virus-associated hemophagocytic syndrome; NK, natural killer cell; HIV, human immunodeficiency virus.

EBV-associated diseases generally show viral gene expression limited to one of three patterns of latency (Cohen, J .I. 2000 *N Engl J Med* **343:**481-92). In the first pattern of latency, only EBNA1 is expressed. In the second pattern of latency, EBNA1, LMP-1, and LMP-2 are expressed. In the third pattern of latency, all the latency genes are expressed. Thus, EBV-associated malignancies in which LMP-1 is expressed include nasopharyngeal carcinoma, T-cell lymphoma, Hodgkin's disease, and lymphoproliferative disease-associated lymphoma, and certain non-Hodgkin's lymphomas.

EBV-associated lymphomas express LFA-1 on their surface, which is important for growth of EBV-transformed B cells. KSHV-infected cells encode structural homologs to EBV LMP1 (Glenn, M. *et al.* 1999 *J Virol* **73:**6953-63). Other virus-associated tumors use LFA-1/ICAM interactions, e.g., HTLV-1 associated adult T cell leukemia/lymphoma (Tanaka, Y. *et al.* 1998 *Blood* **91:**3909-19; Tanaka, Y. 1999 *Leuk Lymphoma* **36:**15-23; and Ohnita, K. *et al.* 2002 *Cancer* **94:**1507-16). Also, hepatitis B and hepatitis C inflammation of the liver (which is associated with severity of hepatitis and is likely associated with tumor formation) is related to LFA-1/ICAM interactions (Doi, T. *et al.* 1994 *J Gastroenterol* **29:**164-71; Banner B.F. *et al.* 1997 *Virchows Arch* **431:**181-87). Statins and other compounds that inhibit the activity of LFA-1 are envisioned as being useful in the treatment or prevention of EBV-associated (or herpes virus-associated or other virus-associated) tumors expressing LFA-1 and transforming proteins, for example, nasopharyngeal carcinoma, Hodgkin's disease, lymphoproliferative disease, non-Hodgkin's lymphoma, and T-cell lymphoma, EBV-associated B-cell lymphoma, Kaposi's sarcoma-associated herpesvirus-associated tumor, HTLV-1 associated adult T cell leukemia/lymphoma, and hepatitis B and hepatitis C associated inflammation and/or carcinoma.

Active Agent and Carrier

The term HMG-CoA reductase inhibitor is intended to include all pharmaceutically acceptable salt, ester and lactone forms of compounds which have HMG-CoA reductase inhibitory activity, and therefore the use of such salts, esters and lactone forms is included within the scope of this invention.

Compounds which have inhibitory activity for HMG-CoA reductase can be readily identified by using assays well-known in the art.

Ester derivatives of the described compounds may act as prodrugs which, when absorbed into the bloodstream of a warm-blooded animal, may cleave in such a manner as to release the drug form and permit the drug to afford improved therapeutic efficacy.

Herein, the term "pharmaceutically acceptable salts" shall mean non-toxic salts of the compounds employed in this invention which are generally prepared by reacting the free acid with a suitable organic or inorganic base. Examples of such salts include, but are not limited to, acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynapthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, palmoate, palmitate, panthothenate, phosphate/diphosphate, polygalacturonate, potassium, salicylate, sodium, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, valerate.

Effective amounts of the HMG-CoA reductase inhibitors (RIs) are suitable for use in the compositions and methods of the present invention. A therapeutically effective amount is intended to mean the amount of a compound sufficient to result in regression or resolution of a neoplasm or tumor burden, or in diminution or amelioration of symptoms or signs of said neoplasm or tumor burden. A prophylactically effective amount is intended to mean the amount of a compound sufficient to prevent or reduce the risk of onset or development of a neoplasm or tumor burden.

The dosage regimen utilizing an HMG-CoA RI is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the subject; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the subject; and the particular compound or salt or ester thereof employed. A consideration of these factors is well within the purview of the ordinarily skilled clinician for the purpose of determining the effective amounts of the drug needed to prevent or treat the risk of occurrence of the condition sought to be prevented or treated. The ordinarily skilled clinician will be able to titrate the subject to the appropriate amount of lipid altering agent such as an HMG-CoA RI which, when taken on a daily basis, will allow the patient to reach their goal.

The term "patient" or "subject" includes mammals, especially humans, who take a lipid altering agent for any of the uses described herein. Administration of the lipid altering agent to the subject includes both self-administration and administration to the subject by another person.

Dosage information for HMG-CoA RI's is well known in the art, since several are marketed in the U.S. In particular, the daily dosage amounts of the HMG-CoA reductase inhibitor may be the same or similar to those amounts which are employed for anti-hypercholesterolemic treatment and which are described in the Physicians' Desk Reference (PDR). Dosage amounts will vary depending on the potency of the specific HMG-CoA reductase inhibitor used as well as other factors as noted above. An HMG-CoA RI which has sufficiently greater potency may be given in sub-milligram daily dosages.

As examples, the daily dosage amount for simvastatin may be selected from 10, 20, 40, 60 or 80 mg/day; for lovastatin, 20, 40, 60, or 80 mg/day; for fluvastatin, 20, 40, 60 or 80 mg/day; for atorvastatin, 10, 20, 40, 60 or 80 mg/day; for cerivastatin, 0.2, 0.3, or 0.4 mg/day; for rosuvastatin, 5, 10, 20, 40, 60 or 80 mg/day; for pitavastatin 4 mg/day. In an alternative embodiment, the dose of a statin is 40 times the dose used against cholesterol. Oral administration may be in single or divided doses of two, three, or four times daily, although a single daily dose of the HMG-CoA RI is preferred.

Pharmaceutical formulations for HMG-CoA reductase inhibitors are well-known to those skilled in the art, as evidenced by the information provided in the Physicians' Desk Reference (PDR). While the HMG-CoA reductase inhibitor can be administered orally or parenterally, oral dosing is preferred.

For example, the active agents employed in the instant methodology can be administered in such oral forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. Oral formulations are preferred. The instant invention includes the use of oral rapid-release as well as time-controlled release pharmaceutical formulations.

The active drug can be administered in admixture with pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices. See, for example, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with a non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, modified sugars, modified starches, methyl cellulose and its derivatives, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and other reducing and non-reducing sugars, magnesium stearate, steric acid, sodium stearyl fumarate, glyceryl behenate, calcium stearate and the like. For oral administration in liquid form, the drug components can be combined with non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring and flavoring agents can also be incorporated into the mixture. Stabilizing agents such as antioxidants (BHA, BHT, propyl gallate, sodium ascorbate, citric acid) can also be added to stabilize the dosage forms. Other suitable components include gelatin, sweeteners, natural and synthetic gums such as acacia, tragacanth or alginates, carboxymethylcellulose, polyethylene glycol, waxes and the like.

The active drug can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Active drug may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. Active drug may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinyl-pyrrolidone, pyran copolymer, polyhydroxy-propyl-methacrylamide-phenol, polyhydroxy-ethyl-aspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, active drug may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross linked or amphipathic block copolymers of hydrogels.

Simvastatin Induces Apoptosis of Epstein-Barr Virus (EBV)-Transformed Lymphoblastoid Cell Lines and Delays Development of EBV-Lymphomas

Simvastatin and pravastatin are inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase, and are used as antihypercholesterolemia drugs. Simvastatin, but not pravastatin, binds to the I (inserted)-domain of leukocyte function antigen-1 (LFA-1) and inhibits the

function of LFA-1, including adhesion and co-stimulation of lymphocytes. Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) express high levels of LFA-1 on their surface and grow in tight clumps. Here we show that simvastatin (2 μM) inhibits clump formation and induces apoptosis of EBV-transformed LCLs. The apoptosis-inducing effect of simvastatin depends on binding to the inserted domain of LFA-1. Simvastatin, but not pravastatin, dissociates EBV latent membrane protein 1 from lipid rafts of LCLs resulting in down-regulation of nuclear factor-κB activity and induction of apoptosis. Analysis of multiple EBV-positive and negative cell lines indicated that both LFA-1 and EBV latent membrane protein-1 expression were required for simvastatin's effects. Administration of simvastatin to severe combined immunodeficiency (SCID) mice followed by inoculation with LCLs resulted in delayed development of EBV-lymphomas and prolonged survival of animals. This is the first report in which a drug that targets LFA-1 has been used to kill B cell lymphomas. These data indicate that simvastatin, other LFA-1 inhibiting statins, and LFA-1 inhibiting statin-derived and statin-like compounds, are useful for treatment or prevention of EBV-associated (or herpes virus-associated or other virus-associated) tumors, including lymphomas and carcinomas, expressing LFA-1 and transforming proteins.

Simvastatin inhibits clump formation and induces apoptosis of LCLs

LCLs and Burkitt lymphoma cells that express each of the EBV nuclear antigens (EBNAs), latent membrane proteins (LMPs), and LFA-1 grow in tight clumps. Binding of anti-LFA-1 antibody TS1/22 to the I domain of LFA-1 inhibits clumping of phorbol myristate acetate-stimulated LCLs after 18 h (Rothlein, R. & Springer, T.A. 1986 *J Exp Med* **163**:1132-1149). To determine whether simvastatin affects clumping of unstimulated LCLs, an LCL (12A1) was treated with various concentrations of the drug. Five days after the addition of simvastatin, LCL clumps broke apart in wells treated with $\geq$ 2 μM simvastatin, whereas clumps remained in wells treated with $\leq$ 1 μM simvastatin (Fig. 5A). Dissociation of clumps was also observed in other LCLs (6B10 and 295H) and in Mutu-3 cells. Cell viability assays were performed on various cell lines expressing different levels of LFA-1, ICAM-1, and LMP-1 (Table 3 and Figure 9). Cell proliferation assays showed that simvastatin decreased the number of viable cells in a dose-dependent manner (Fig. 5B). A loss in viability of LCLs was induced by 0.5 μM simvastatin, whereas 2 μM simvastatin was required to reduce viability of other cells. Trypan blue staining showed that LCLs 12A1 and 295H had >50% reduction in viability with 2 μM simvastatin, whereas Akata and

P3HR-1 cells required higher concentrations of the drug (4 μM) to achieve >50% reduction in viability (Fig. 5C). The number of dead cells began to increase 5 days after addition of simvastatin at the time when clumps started to dissociate. Propidium iodide (PI) staining followed by flow cytometry was also used to determine cell viability with 2 μM simvastatin. Cells expressing LMP-1 such as LCLs (12A1, 6B10 and 295H) and Mutu-3 showed low (<50%) viability after culture in 2 μM simvastatin for 7 days (Fig. 5D). Thus, incubation of cells with 2 μM simvastatin for 5 days inhibits clump formation and induces death in cells expressing LMP-1 (Table 3). Two other statins, lovastatin and atorvastatin, in addition to simvastatin kill EBV-transformed cells (Fig. 10A, B).

Table 3. Protein expression, activation of NF-κB, and cell death induced by simvastatin in cell lines.

Cell line	Description	Clumping formation	LFA-1*	ICAM-1*	EBV**	LMP-1***	NF-κB***	Cell death by simva-statin****
12A1	LCL	++	++	++	+	+++	++	+
6B10	LCL	++	++	++	+	+++	+++	+
295H	LCL	+	+	++	+	+++	+++	+
Mutu-1	B cell line (BL)	-	-	-	+	-	-	-
Mutu-3	B cell line (BL)	+	+	++	+	+	+	+
P3HR-1	B cell line (BL)	-	++	++	+	-	+	-
Akata	B cell line (BL)	-	-	-	+	-	+	-
BCBL-1	HHV-8 positive cell	-	-	+	-	-	-	-
BJAB	B cell line (BL)	-	+	++	-	-	-	-
II-23	T cell line	-	++	+	-	-	+	-
Jurkat	T cell line	-	-	+	-	-	-	-

*Expression levels of LFA-1 and ICAM-1 were determined with flow cytometry (Fig 9).
EBV-positive cells lines are indicated in Miller, G. *et al.* 1974 *PNAS USA* **71:4006-10; Takada, K. *et al.* 1991 *Virus Genes* **5**:147-56; Gregory, C.D. *et al.* 1990 *J Gen Virol* **71**:1481-95; Renne, R. *et al.* 1996 *Nat Med* **2**:342-6; Menezes, J. *et al.* 1975 *Biomedicine* **22:** 276-84; Schneider, U. *et al.* 1977 *Int J Cancer* **19**:621-6; Ware, C.F. *et al.* 1986 *Lymphokine Res* **5:** 313-24.
***Expression level of LMP-1 and constitutive activation of NF-κB were determined by immunoblot and gel shift assay, respectively (Fig. 9).
****Cell death induced by 2 μM simvastatin is defined as >50% cell death by PI staining (Fig. 5D).
BL: Burkitt lymphoma; HHV-8: human herpesvirus 8.

Some statins induce apoptosis of certain tumor cells *in vitro* (Wong, W. W. et al. 2002 *Leukemia* **16**:508-19). PI staining showed that treatment of LCLs with simvastatin for

5 days induced a dose dependent increase in fragmented DNA that was smaller than G0-G1 DNA (2n), indicative of apoptosis (Darzynkiewicz, Z. et al. 1992 *Cytometry* **13**:795-808) (Fig. 5E). Terminal deoxytransferase-mediated dUTP nick end labeling (TUNEL) assay confirmed that simvastatin induced DNA fragmentation in LCLs (Fig. 5F). Gel electrophoresis showed that simvastatin induced DNA fragments in LCLs resulting in formation of a DNA ladder (Fig. 5G). Thus, simvastatin induces apoptosis in LCLs.

The apoptosis-inducing effect is specific for simvastatin and dependent on binding to the I domain of LFA-1

To determine whether loss of cell viability of LCLs occurs with a statin with different binding properties than simvastatin, LCLs were incubated with pravastatin, which does not bind to LFA-1 (Weitz-Schmidt, G. et al. 2001 *Nat Med* 7:687-92). Pravastatin had little effect on viability of LCL 12A1 or 6B10 (Fig. 6A and B). Anti-LFA-1 monoclonal antibody TS1/22 and soluble ICAM-1 (sICAM-1) bind to the I domain of LFA-1 (Huang, C. & Springer, T.A. 1995 *J Biol Chem* **270**:19008-16.; Marlin, S.D. et al. 1990 *Nature* **344**:70-2), which is the site on LFA-1 targeted by simvastatin (Weitz-Schmidt, G. et al. 2001 *Nat Med* 7:687-92). Anti-LFA-1 TS1/22 antibody bound to LFA-1 on the cell surface at a 1:100 dilution by flow cytometry (Fig. 9); however, the antibody did not affect viability of 12A1 cells at concentrations up to 1:16 (Fig. 6C). sICAM-1 used at concentrations of 5 μg/ml that block rhinovirus infection (Marlin, S. D. *et al.* 1990. *Nature* **344:** 70-2) did not affect viability of LCLs 12A1 and 295H (Fig. 6D). Thus, antibody or another ligand that binds to the I domain of LFA-1 does not induce death of LCLs.

Antibody to LFA-1 (TS1/22) has been shown to induce signal transduction in lymphocytes (Perez, O.D. et al 2003 *Nat Immunol* **4**:1083-1092). Therefore, pretreatment of LCLs with the antibody might inhibit the effects of simvastatin. Pretreatment of cells with anti-LFA-1 antibody TS1/22 blocked the ability of simvastatin to dissociate clumps of LCLs (Fig. 6E) and to reduce viability of LCLs (Fig. 6F).

Simvastatin, but not pravastatin, dissociates LMP-1 from lipid rafts, and reduces activation of NF-κB

In vitro assays indicate that cholesterol depletion by high doses of statins disrupts lipid rafts and alters the localization and function of proteins in lipid rafts on the cell membrane (Gubina, E. et al. 2002 *Blood* **99**:2518-25; Hansen, G.H. et al. 2000 *J Biol Chem* **275**:5136-42; Simons, K. & Toomre, D. 2000 *Nat Rev Mol Cell Biol* **1**:31-9). EBV LMP-1

localizes in lipid rafts and constitutively activates NF-κB via tumor necrosis factor receptor-associated factors (TRAF) (Mosialos, G. et al. 1995 *Cell* **80**:389-99; Higuchi, M. et al. 2001 *PNAS USA* **98**:4675-80; Kaykas, A. et al. 2001 *EMBO J* **20**:2641-54). Therefore, we postulated that simvastatin may affect localization of LMP-1 in lipid rafts and impair signal transduction by LMP-1. To determine whether simvastatin alters localization of LMP-1, we treated LCLs with simvastatin and performed immunofluorescence assays for the viral protein. LMP-1 localized to large punctate structures in LCLs in the absence of simvastatin (Fig. 7A). After treatment with simvastatin for 3 or 7 days, LMP-1 showed a fine granular pattern in most of the cells that was much fainter and more diffuse than in untreated cells. In contrast, LMP-1 maintained its large punctate structures in cells treated with pravastatin. To further examine the effect of statins on LMP-1 localization, cell extracts were fractionated by using centrifugation and floatation in sucrose gradients (Higuchi, M. et al. 2001 *PNAS USA* **98**:4675-80). Lyn localizes to lipid rafts, whereas CD71 is in the soluble fraction (Higuchi, M. et al. 2001 *PNAS USA* **98**:4675-80). Immunoblotting showed that lipid raft fractions (fractions 3 and 4) contained the highest concentrations of LMP-1 in untreated or pravastatin-treated LCLs. At 2 μM simvastatin, the highest concentrations of LMP-1 shifted to soluble fractions (fractions 7-12), but Lyn remained in lipid rafts (fractions 3 and 4) (Fig. 7B). Quantitative analysis showed that 47% of LMP-1 was localized in lipid rafts of untreated cells; after treatment with simvastatin for 3 days, only 7% of LMP-1 was located in rafts. These data indicate that simvastatin, but not pravastatin, alters the localization of LMP-1 in the cell, and dissociates LMP-1 from lipid rafts.

Each of the LCLs used in the present study showed constitutive activation of NF-κB (Fig. 9). Electrophoretic mobility-shift assays showed that treatment of LCLs with simvastatin (2 μM or 4 μM) for 3 days reduced the level of activated NF-κB, whereas pravastatin (8 μM) did not reduce NF-κB (Fig. 7C). Cell viability at 3 days ranged from 80-90% in cells treated with either statin or in untreated cells. Because inhibition of NF-κB induces apoptosis of LCLs (Cahir-McFarland, E. D. et al. 2000 *PNAS USA* **97**:6055-60), our results indicate that reduction of NF-κB by simvastatin results in induction of apoptosis in LCLs.

Simvastatin delays the onset of EBV-lymphomas and prolongs survival in SCID mice inoculated with EBV-transformed LCLs

Because simvastatin induced apoptosis of LCLs, we tested the effect of the drug on SCID mice with EBV-lymphomas. Simvastatin (250 mg/kg/day) was given orally to SCID mice beginning 3 days before intraperitoneal inoculation with EBV-transformed LCLs. Control animals did not receive simvastatin. Four or six weeks later, simvastatin was discontinued because of side effects from the drug (Table 4) and mice were followed for development of tumors. Seven weeks after inoculation with LCLs, 80% of mice in the control group that received $0.25x10^6$ cells developed ascites, whereas none of the animals treated with simvastatin that received the same number of cells had ascites (Fig. 8A). Nine weeks after inoculation, more than 70% of the control mice were dead of lymphoma, whereas all mice treated with simvastatin that received the same number of cells were alive, although a few had ascites. Mice pretreated with simvastatin that received different doses of LCLs all survived significantly longer compared to mice that did not receive the drug (Table 4). At autopsy all of these control mice had immunoblastic lymphomas (Fig. 8A). Flow cytometry showed that expression of LFA-1 in tumor cell ascites was reduced in mice treated with simvastatin compared with mice that did not receive the drug. In a separate series of experiments using LCL 6B10, mice that received simvastatin 3 days before inoculation with LCLs (pretreatment group) were compared with mice that received the drug 7 days after inoculation (treatment group). Mice in the pretreatment group survived significantly longer ($p<0.04$) compared with animals in the control group; however, the difference in survival was not significant for animals in the treatment group versus the control group ($p=0.2135$) (Table 4, Fig. 8B).

Table 4. SCID mouse experiments with simvastatin.

Group	LCL	Cell number	No. of mice*	Time of Therapy**	Simvastatin days (mg/kg/day)	50% survival (days)	Day of death (range in days)	*p* (logrank) ***
1	12A1	$0.25x10^6$	6	Pretreatment	-3 to +28 (250)	81	70->105	.0496
2		$0.25x10^6$	10	No treatment	- (0)	56	45-105	
3		$1 x10^6$	6	Pretreatment	-3 to +28 (250)	56	53->105	.0350
4		$1 x10^6$	12	No treatment	- (0)	53	48-56	
5		$4 x10^6$	10	Pretreatment	-3 to +28 (250)	56	49-57	<.0001
6		$4 x10^6$	12	No treatment	- (0)	46	43-50	
7	6B10	$1 x10^6$	10	Pretreatment	-3 to +35 (250)	>100	64->100	.0375
8		$1 x10^6$	11	Treatment	+7 to +35 (250)	85	76->100	
9		$1 x10^6$	11	No treatment	- (0)	71	54->100	.2135

*Numbers of mice analyzed. At four weeks, 6 animals receiving 0.25×10^6 of LCL 12A1 and simvastatin, 6 receiving 1×10^6 of 12A1 and simvastatin, and 2 animals receiving 4×10^6 12A1 and simvastatin died or were sacrificed because of simvastatin side effects. These included severe weight loss, hunched body, or red swollen eyes. Autopsies showed no evidence of ascites or lymphomas in these mice and they were not included in the table and were excluded from the analysis.

**Time of therapy: Animals received simvastatin 3 days before (pretreatment) or 7 days after (treatment) inoculation with LCLs.

***Significance: *p* values indicated are for mice treated with simvastatin versus mice not treated with the drug for each cell number.

EXAMPLE 1

Cell culture and viability assay

Three EBV-transformed LCLs, 12A1, 6B10, and 295H, EBV-positive Burkitt lymphoma cell lines: P3HR-1 (Miller, G. et al. 1974 *PNAS USA* **71**:4006-10), Akata (Takada, K. et al. 1991 *Virus Genes* **5**:147-56), Mutu-1 (Gregory, C. D. et al. 1990 *J Gen Virol* 71:1481-95), and Mutu-3 (Gregory, C. D. et al. 1990 *J Gen Virol* **71**:1481-95), a human herpesvirus-8-positive EBV-negative primary effusion lymphoma cell line, BCBL-1

(Renne, R. et al. 1996 *Nat Med* **2**:342-6), an EBV-negative Burkitt lymphoma cell line, BJAB (Menezes, J. et al. 1975 *Biomedicine* **22**:276-84), and EBV-negative T cell lines: Jurkat (Schneider, U. et al. 1977 *Int J Cancer* **19**:621-6) and II-23 (Ware, C.F. et al. 1986 *Lymphokine Res* **5**:313-24) cells, obtained from Carl Ware, La Jolla Institute for Allergy and Immunology, San Diego, CA, were tested. For cell proliferation and viability assays, $2x10^4$ cells per ml were cultured in 12 or 24-well plates for 5-7 days. Cell viability was assessed with the Cell Proliferation Kit II (XTT) (Roche Molecular Biochemicals, Indianapolis, IN), trypan-blue or propidium iodide (PI) staining. For PI staining, cells were washed with PBS, PI (5 μg/ml) was added, cells were washed, and fluorescent intensity was assessed with flow cytometry. Percent cell death was determined by the ratio of PI-positive cells to all gated cells.

Reagents and antibodies

Simvastatin and pravastatin (Calbiochem, San Diego, CA) were converted to their open acid forms before use *in vitro* (Liu, L. et al. 1999 *J Biol Chem* **274**:33334-40). Soluble ICAM-1 was purchased from R&D Systems, Inc. (ADP4, Minneapolis, MN). LFA-1 antibodies TS1/22 [American Type Culture Collection (ATCC), Manassas, VA] (Huang, C. & Springer, T.A. 1995 *J Biol Chem* **270**:19008-16) and G25.2 (BD Pharmingen, San Diego, CA) were used as primary antibodies and fluorescein isothiocyanate (FITC)-conjugated $F(ab')_2$ fragment of goat anti-mouse immunoglobulin (Caltag, Burlingame, CA) was used as the secondary antibody for immunofluorescence and flow cytometry. TS1/22 antibody was obtained from hybridoma cells.

Lipid raft studies

Cells (2×10^7) were lysed at 4°C in 1 ml of 1% or 0.2% Triton X-100 in MNE buffer (25 mM Mes, pH 6.5/150 mM NaCl/5 mM EDTA) containing proteinase inhibitor cocktail (Roche Diagnostic, Indianapolis, IN), subjected to 10 strokes of tight-fitting Dounce, and mixed with 1 ml of 80% sucrose in MNE buffer. The lysate was transferred to a centrifuge tube and overlaid with 2 ml of 30% sucrose and 1 ml of 5% sucrose. After centrifugation for 18 h at 200,000 × *g*, 0.4-ml fractions were collected from the top of the gradient. Fractions were mixed with 0.4 ml of 2× SDS sample buffer. The pellet fraction was suspended in 0.4 ml of 1% Triton X-100 in MNE buffer, mixed with SDS sample buffer, and sonicated. Twenty microliters of the individual fractions was subjected to SDS/PAGE, transferred to nitrocellulose membranes, and probed with indicated antibodies.

For immunoprecipitation, fractions were mixed with 0.4 ml of 120 mM octyl β-glucoside in MNE buffer. The pellet fraction was suspended in 0.4 ml of 1% SDS, solubilized by sonication, and mixed with 0.4 ml of 10% Triton® X-100 in MNE buffer (Higuchi, M. et al. 2001 *PNAS USA* **98**:4675-80). Immunoblotting was performed by using anti-LMP-1 monoclonal antibody (S-12, BD Pharmingen), anti-CD71 monoclonal antibody (Zymed Laboratories, South San Francisco, CA), and anti-Lyn monoclonal antibody (Santa Cruz, Santa Cruz, CA).

Nuclear extraction and electrophoretic mobility shift assays

Nuclear extracts were prepared from $1x10^7$ cells as described previously (Cahir-McFarland, E. D. et al. 2000 *PNAS USA* **97**:6055-60). Briefly, cells were swollen on ice in 1 ml of hypotonic buffer A [20 mM Hepes, pH 7.0/10 mM KCl/1 mM $MgCl_2$, 0.5 mM DTT, 0.1% Triton X-100, 1x Complete® proteinase inhibitor cocktail (Roche Diagnostic)], homogenized by douncing, and fractionated at 13,000 × g. The pellet was resuspended in buffer A in 50 μl. Activation of NF-κB activation was performed using 5 μg of nuclear extract in the gel shift assay system (Promega, Madison, WI) according to the manufacturer's instructions.

Apoptosis assays

DNA fragmentation by apoptosis was detected by propidium iodide (PI) staining. Apoptotic cells can be recognized by their diminished stainability with DNA specific fluorochromes, such as PI, DAPI, acridine orange (AO), or Hoechst dyes, due to DNA degradation and its subsequent leakage from the cell. Because the degree of DNA leakage can be manipulated by the extent of cell washing after fixation (or permeabilization), it is possible to adjust DNA content of apoptotic cells (position of the "sub-G_1 peak" on the DNA frequency histograms) so that their overlap with cells that do not undergo apoptosis is minimal and separation between these two populations is adequate. To determine the percentage of dead cells (Fig. 5D), cells were washed with PBS, PI (5μg/ml) was added, cells were washed, again, and fluorescent intensity was assessed by flow cytometry. Percent cell death was determined by the ratio of PI-positive cells to all gated cells. For apoptosis assays using PI staining (Fig. 5E), the cells were fixed, suspended in Hank's buffer saline and maintained at 37°C for 20 min prior to staining and flow cytometry (Darzynkiewicz, Z. et al. 1992 *Cytometry* **13**:795-808). Terminal deoxytransferase-mediated dUTP nick end labeling (TUNEL) assays were performed by using the *in situ* cell

death detection kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. DNA ladder formation was performed by extracting DNA from cells using the genomic DNA purification kit (Gentra Systems, Minneapolis, MN) and electrophoresis was performed.

Animal experiments

Simvastatin tablets (Zocor®, Merck & Co. Inc., Whitehouse Station, NJ) were mixed with mouse food at a ratio of 160 mg of simvastatin per 65 g of powdered food. Untreated animals received powdered food without simvastatin. LCLs ($0.25x10^6$, $1x10^6$, or $4x10^6$) LCLs were inoculated intraperitoneally into 8-week old SCID mice. Simvastatin was given either 3 days before (pretreatment group) or 7 days after (treatment group) inoculation of cells and continued until 4-5 weeks after inoculation. Thereafter, powered food without simvastatin was given to all mice, because of the side effects of prolonged high-dose simvastatin. All dead mice were autopsied and examined for the presence of lymphomas.

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While the present invention has been described in some detail for purposes of clarity and understanding, one skilled in the art will appreciate that various changes in form and detail can be made without departing from the true scope of the invention. All figures, tables, and appendices, as well as patents, applications, and publications, referred to above, are hereby incorporated by reference.

WHAT IS CLAIMED IS:

1. A method for treatment or prevention of an EBV-associated (or herpes virus-associated or other virus-associated) tumor, including lymphoma and carcinoma, expressing LFA-1 and transforming proteins comprising administration to a subject in need of such treatment or prevention of an effective amount of simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound.

2. Use of simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound, for the preparation of a medicament for the treatment or prevention of an EBV-associated (or herpes virus-associated or other virus-associated) tumor, including lymphoma and carcinoma, expressing LFA-1 and transforming proteins.

3. The method or use of claim 1 or 2 wherein the simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound is simvastatin.

4. The method or use of claim 1 or 2 wherein the simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound is another LFA-1 inhibiting statin.

5. The method or use of claim 1 or 2 wherein the simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound is an LFA-1 inhibiting statin-derived or statin-like compound.

6. The method or use of claim 1 or 2 wherein the simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound is lovastatin.

7. The method or use of claim 1 or 2 wherein the simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound is atorvastatin.

8. The method or use of claim 1 or 2 wherein the simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound is LFA703 (Novartis), BIRT377 (Boehringer-Ingelheim), or A-286982 (ICOS/Abbott/Biogen).

9. The method or use of claim 1 or 2 wherein the simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound is LFA451.

10. The method or use of claim 1 or 2 wherein the simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound is Compound 1 (Genentech), Compound 3 (Genentech), Compound 4 (Genentech), or Compound 5 (Roche).

11. The method or use of claim 1 or 2 wherein the EBV-associated (or herpes virus-associated or other virus-associated) tumor is a member selected from the group

consisting of nasopharyngeal carcinoma, Hodgkin's disease, lymphoproliferative disease, non-Hodgkin's lymphoma, and T-cell lymphoma.

12. The method or use of claim 1 or 2 wherein the EBV-associated (or herpes virus-associated or other virus-associated) tumor is an EBV-associated B-cell lymphoma.

13. The method or use of claim 1 or 2 wherein the EBV-associated (or herpes virus-associated or other virus-associated) tumor is a Kaposi's sarcoma-associated herpesvirus-associated tumor.

14. The method or use of claim 1 or 2 wherein the administration is to an immunocompromised human.

15. The method or use of claim 1 or 2 wherein the administration is oral.

16. The method or use of claim 1 or 2 wherein the effective amount is from about 0.6 to about 148.5 mg/kg/day.

17. The method or use of claim 1 or 2 wherein the effective amount is from about 2.0 to about 45.0 mg/kg/day.

18. The method or use of claim 1 or 2 further comprising detection of a level of an identifiable tumor marker.

19. The method or use of claim 1 or 2 wherein administration is in conjunction with another treatment modality.

20. The method or use of claim 15 wherein the other treatment modality includes a dose of a chemotherapeutic agent.

21. The method or use of claim 15 wherein the other treatment modality includes a dose of an additional anti-tumor agent.

22. The method or use of claim 17 wherein the additional anti-tumor agent is an NSAID.

23. A method of making a pharmaceutical composition comprising:

a) identifying simvastatin, other LFA-1 inhibiting statin, or LFA-1 inhibiting statin-derived or statin-like compound, as inhibiting growth or inducing death of virus-associated tumor cells or prolonging the survival of experimental animals with an EBV-associated (or herpes virus-associated or other virus-associated) tumor, including lymphoma and carcinoma, expressing LFA-1 and transforming proteins to provide an active agent; and

b) combining said active agent with a pharmaceutically acceptable carrier to provide said pharmaceutical composition.

24. The method of claim 16 wherein the amount of the active ingredient is from about 40 mg to about 3150 mg.

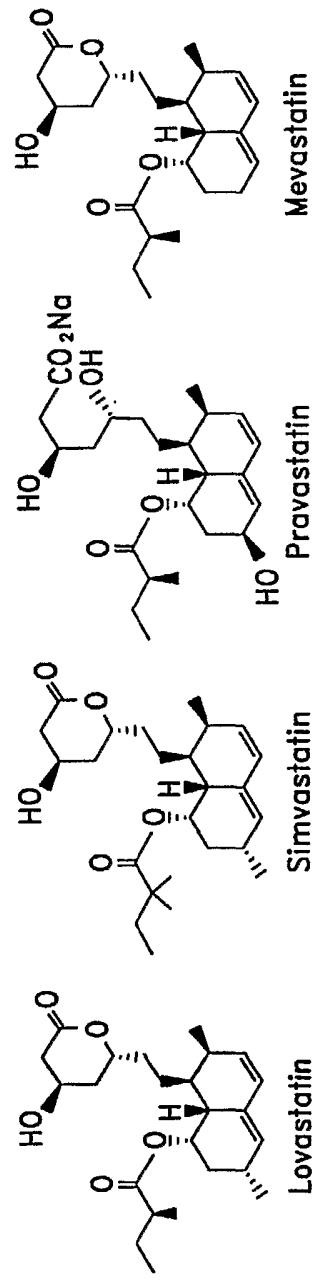

Lovastatin
Simvastatin
Pravastatin
Mevastatin

FIG. 1A

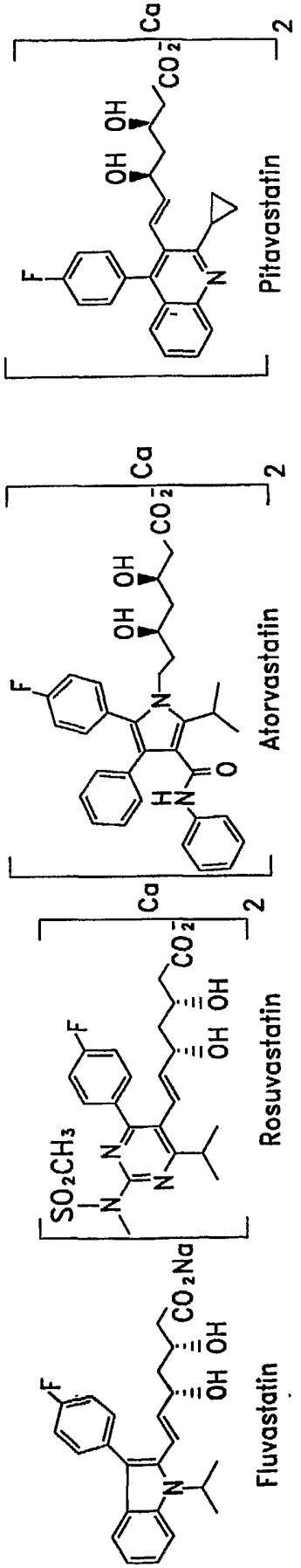

Fluvastatin
Rosuvastatin
Atorvastatin
Pitavastatin

FIG. 1B

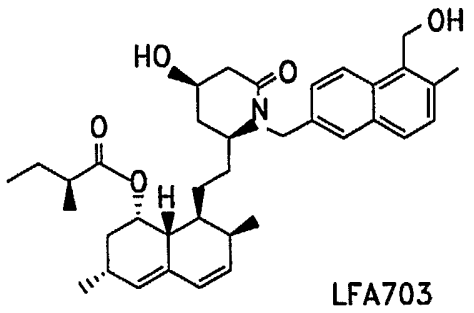

OH
HO
O
N
O
O
H
LFA703

FIG. 1C

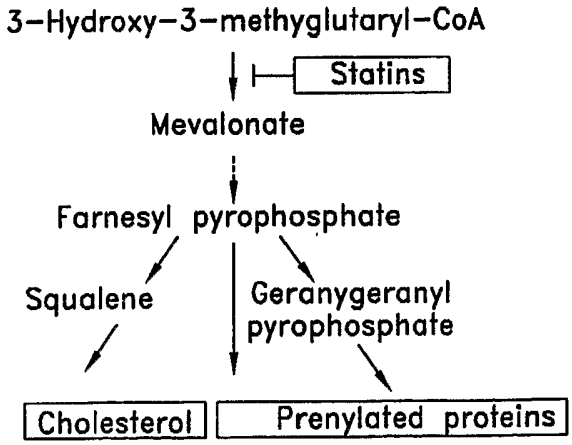

3-Hydroxy-3-methyglutaryl-CoA
Statins
Mevalonate
Farnesyl pyrophosphate
Squalene
Geranygeranyl pyrophosphate
Cholesterol
Prenylated proteins

FIG. 2

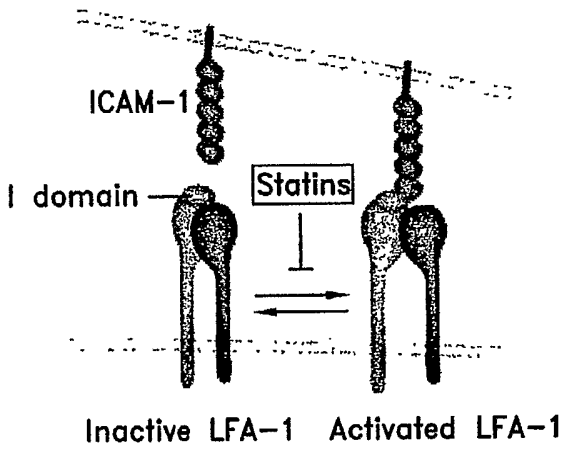

ICAM-1
I domain
Statins
Inactive LFA-1
Activated LFA-1

FIG. 3A

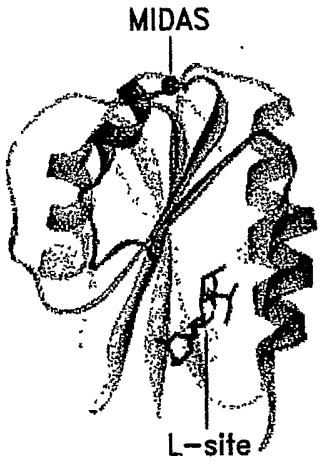
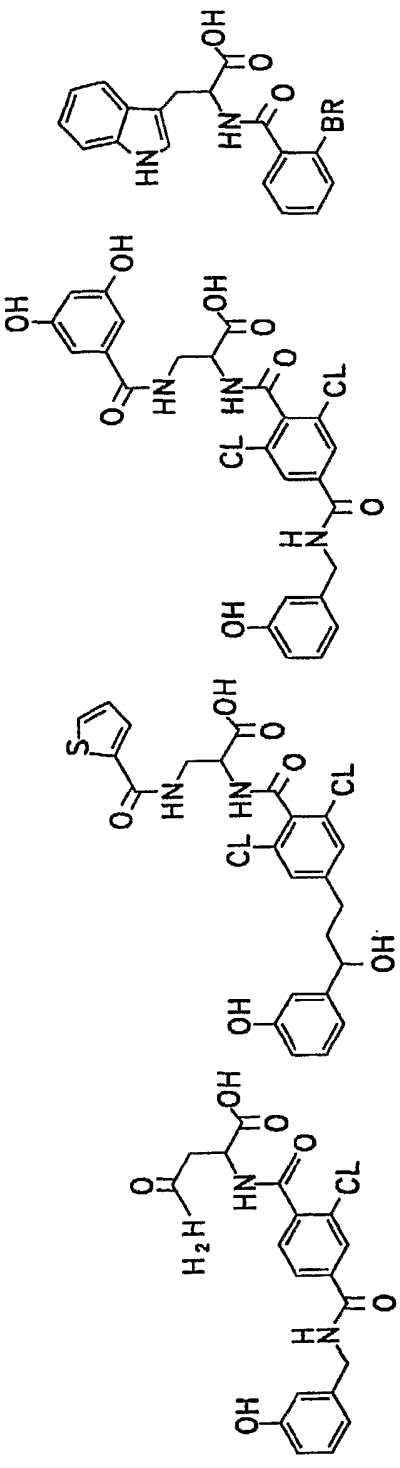

MIDAS
L-site

FIG. 3B

FIG. 4A



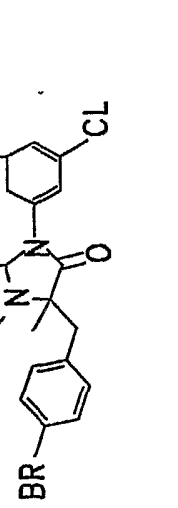
compound #3 — Genentech

compound #4 — Genentech

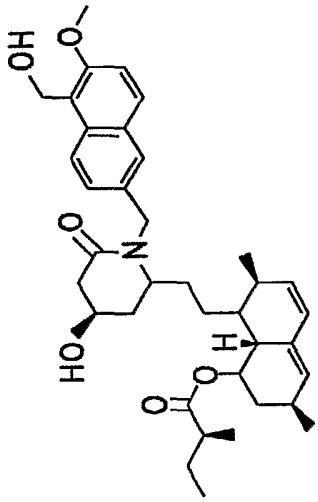
XVA143
compound #5 — Roche

compound #1 — Genentech

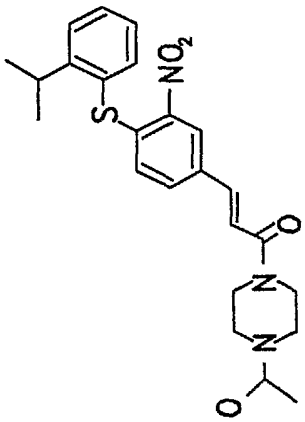
FIG. 4B



BIRT377 — Boehringer-Ingelheim

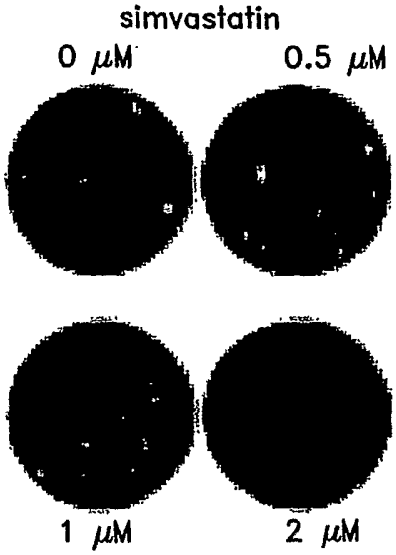


LFA703 — Novartis



A-286982 — ICOS/Abbott/Biogon

FIG. 5A


simvastatin
0 μM
0.5 μM
1 μM
2 μM

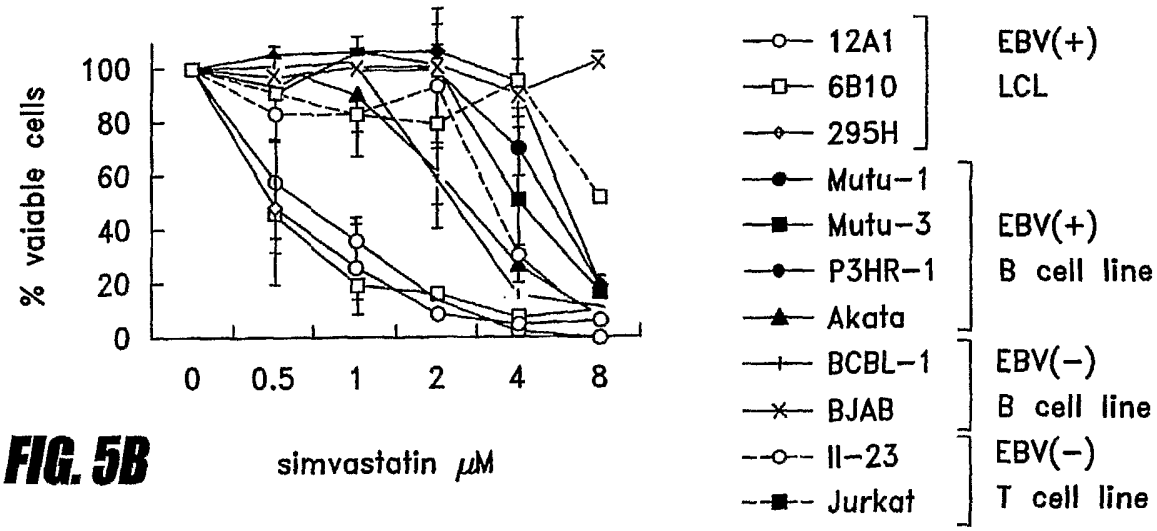

% vaiable cells
100
80
60
40
20
0
0
0.5
1
2
4
8
simvastatin μM
12A1
6B10
295H
EBV(+)
LCL
Mutu-1
Mutu-3
P3HR-1
Akata
EBV(+)
B cell line
BCBL-1
BJAB
EBV(-)
B cell line
II-23
Jurkat
EBV(-)
T cell line

FIG. 5B

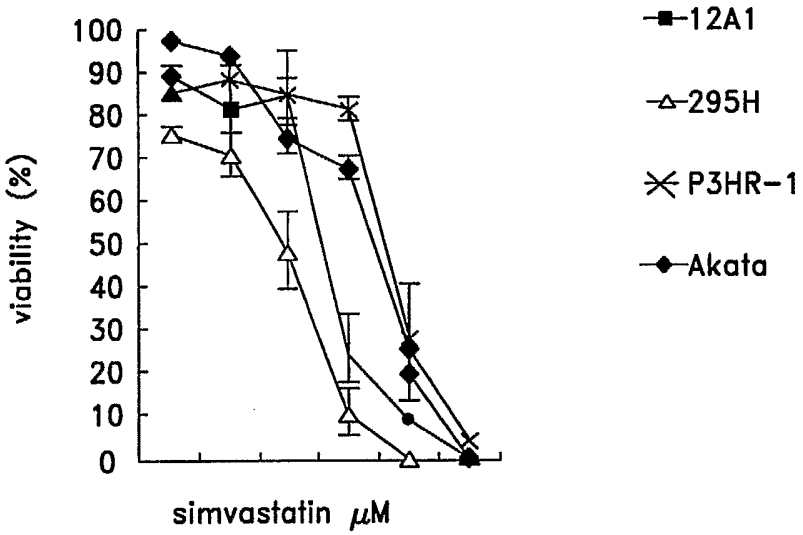

100
90
80
70
60
50
40
30
20
10
0
viability (%)
simvastatin μM
12A1
295H
P3HR-1
Akata

FIG. 5C

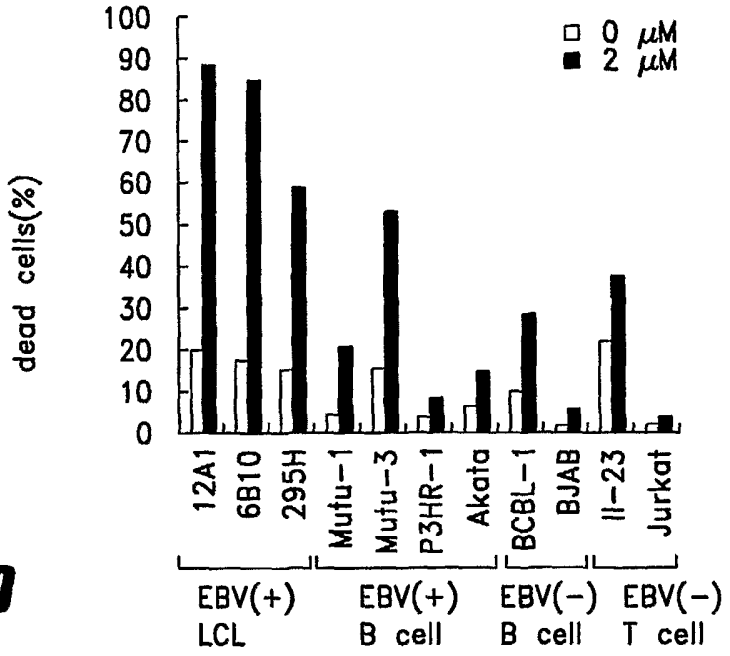

dead cells(%)
100
90
80
70
60
50
40
30
20
10
0
0 μM
2 μM
12A1
6B10
295H
Mutu-1
Mutu-3
P3HR-1
Akata
BCBL-1
BJAB
II-23
Jurkat
EBV(+) LCL
EBV(+) B cell
EBV(-) B cell
EBV(-) T cell

FIG. 5D

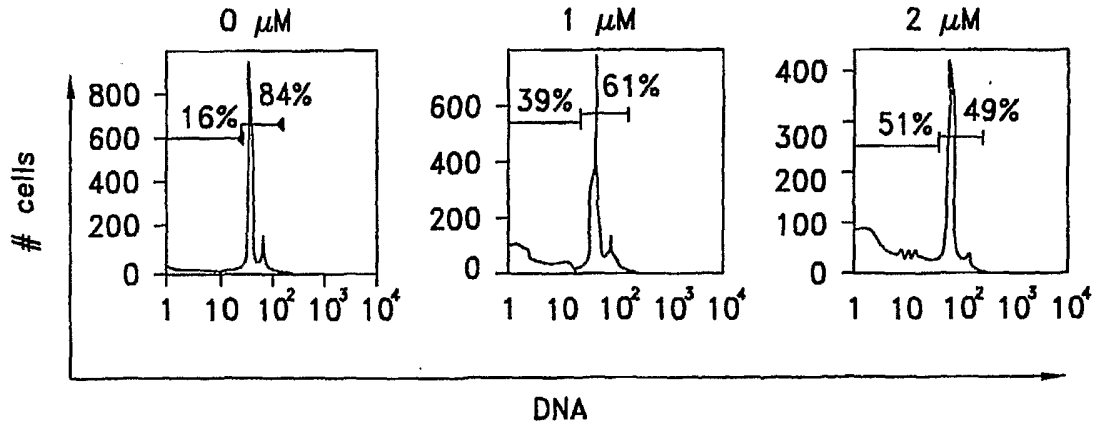
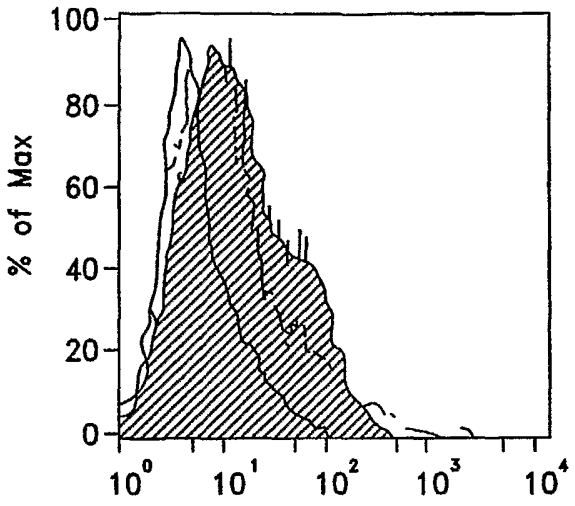

0 μM
1 μM
2 μM
cells
800
600
400
200
0
16%
84%
600
400
200
0
39%
61%
400
300
200
100
0
51%
49%
1 10 10² 10³ 10⁴
DNA

FIG. 5E

FIG. 5F



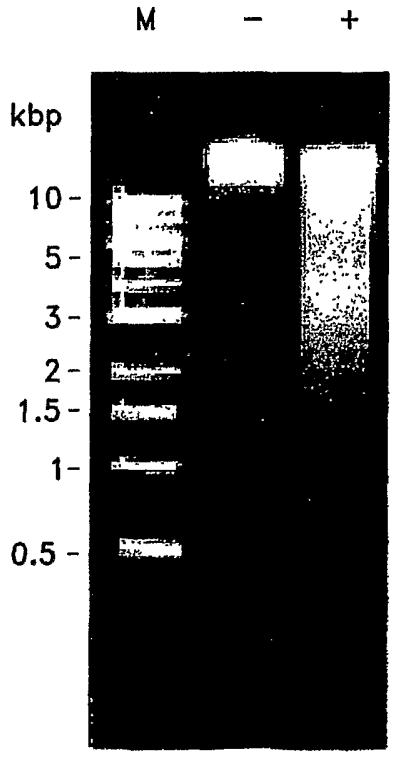


FIG. 5G

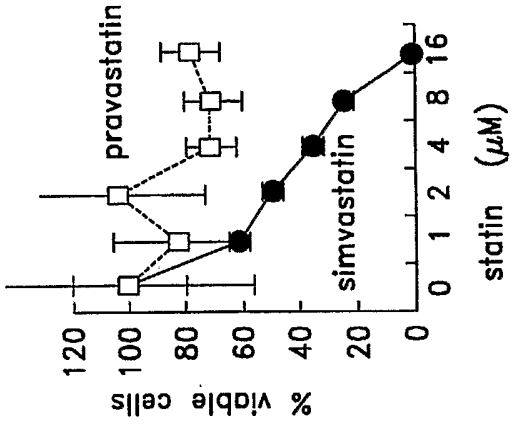

pravastatin
simvastatin
% viable cells
120
100
80
60
40
20
0
0 1 2 4 8 16
statin (μM)

FIG. 6B

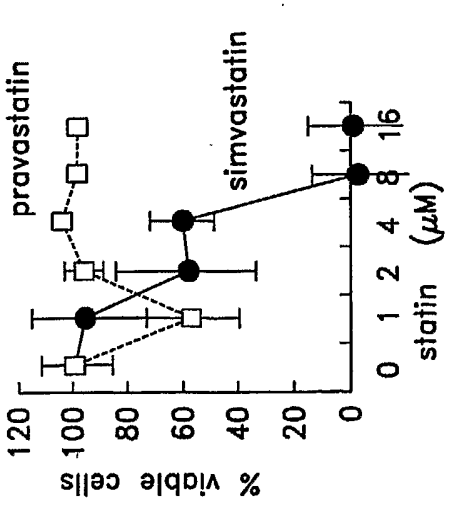

pravastatin
simvastatin
% viable cells
120
100
80
60
40
20
0
0 1 2 4 8 16
statin (μM)

FIG. 6A

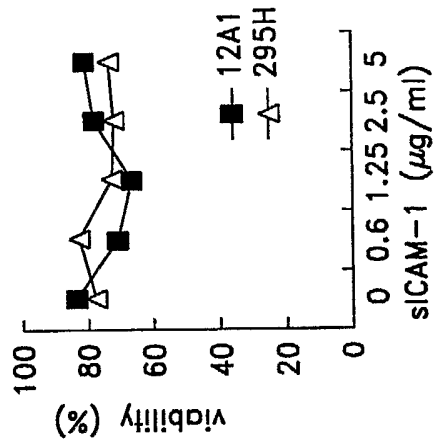

viability (%)
100
80
60
40
20
0
0 0.6 1.25 2.5 5
sICAM-1 (μg/ml)
12A1
295H

FIG. 6D

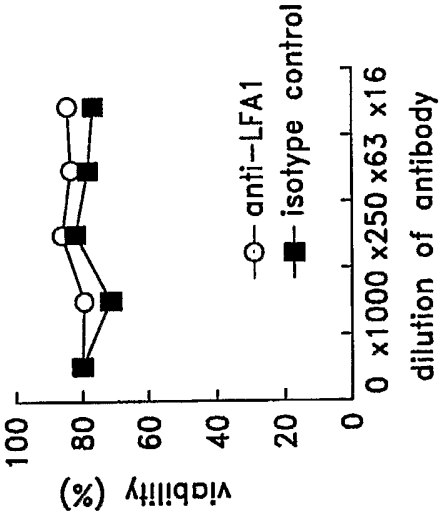

viability (%)
100
80
60
40
20
0
0 x1000 x250 x63 x16
dilution of antibody
anti-LFA1
isotype control

FIG. 6C

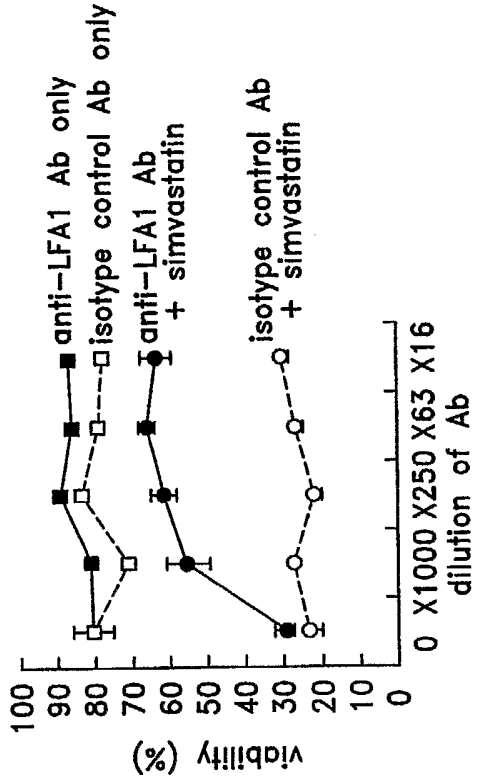

anti-LFA1 Ab only
isotype control Ab only
anti-LFA1 Ab + simvastatin
isotype control Ab + simvastatin
viability (%)
100
90
80
70
60
50
40
30
20
10
0
0 X1000 X250 X63 X16
dilution of Ab

FIG. 6F

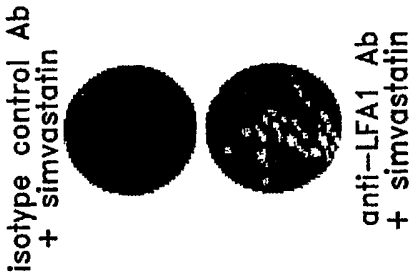

isotype control Ab + simvastatin
anti-LFA1 Ab + simvastatin

FIG. 6E

FIG. 7A

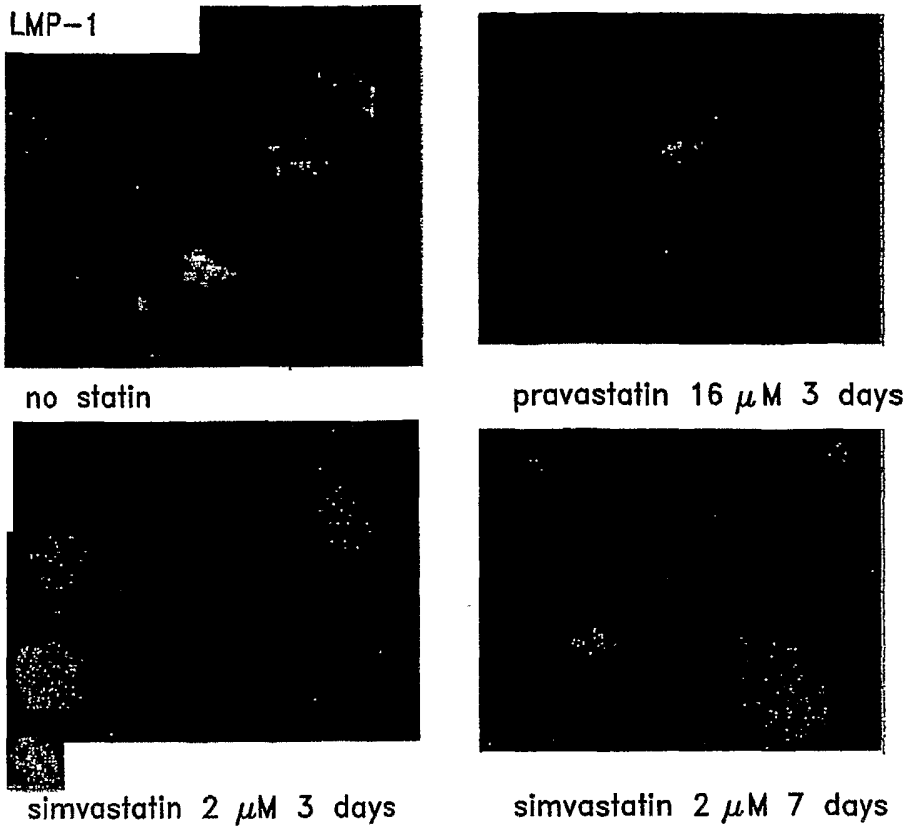
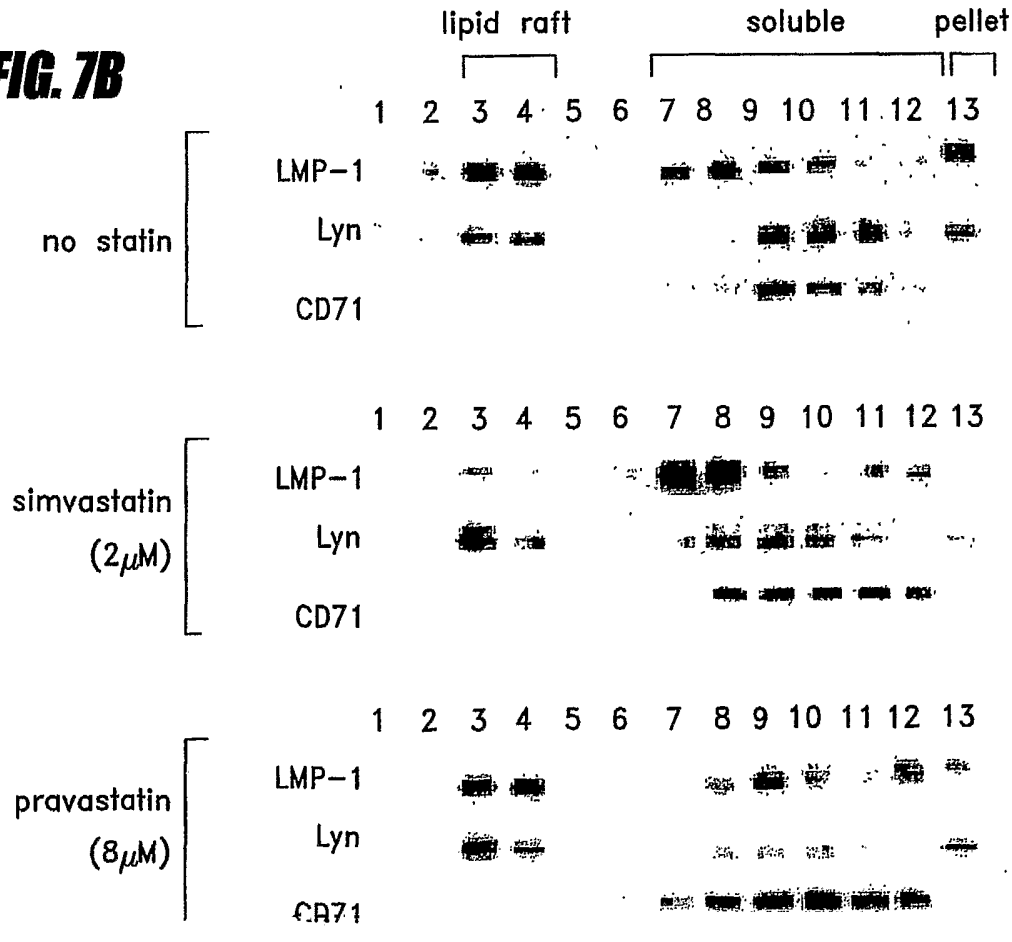

LMP-1
no statin
pravastatin 16 μM 3 days
simvastatin 2 μM 3 days
simvastatin 2 μM 7 days

FIG. 7B


lipid raft
soluble
pellet
1 2 3 4 5 6 7 8 9 10 11 12 13
no statin
LMP-1
Lyn
CD71
simvastatin (2μM)
pravastatin (8μM)

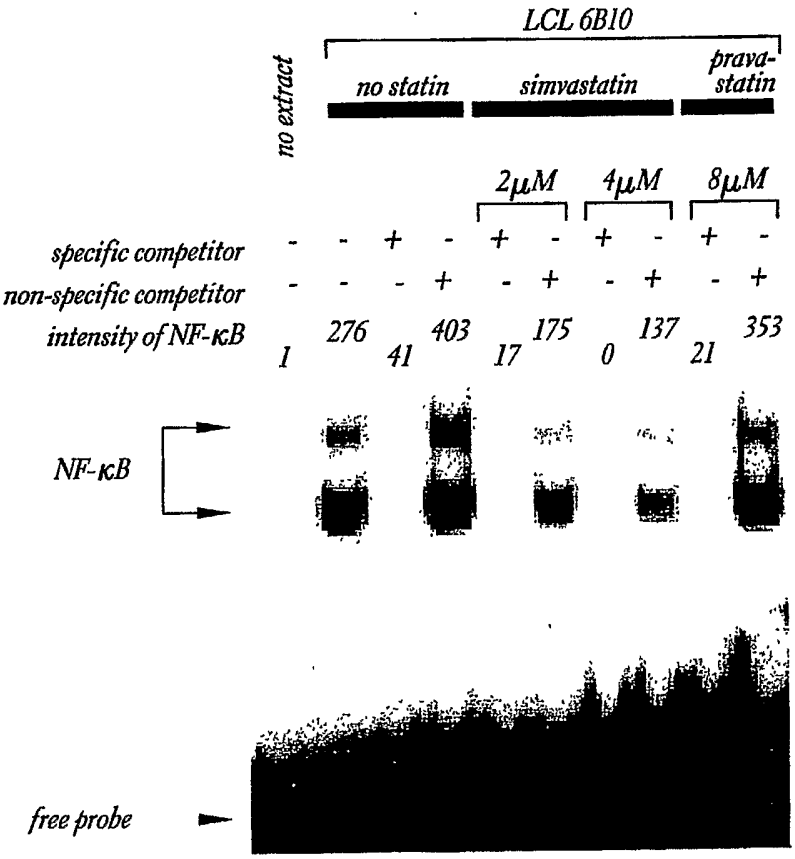

LCL 6B10
no extract
no statin
simvastatin
prava-
statin
2μM
4μM
8μM
specific competitor
non-specific competitor
intensity of NF-κB
1
276
41
403
17
175
0
137
21
353
NF-κB
free probe

FIG. 7C

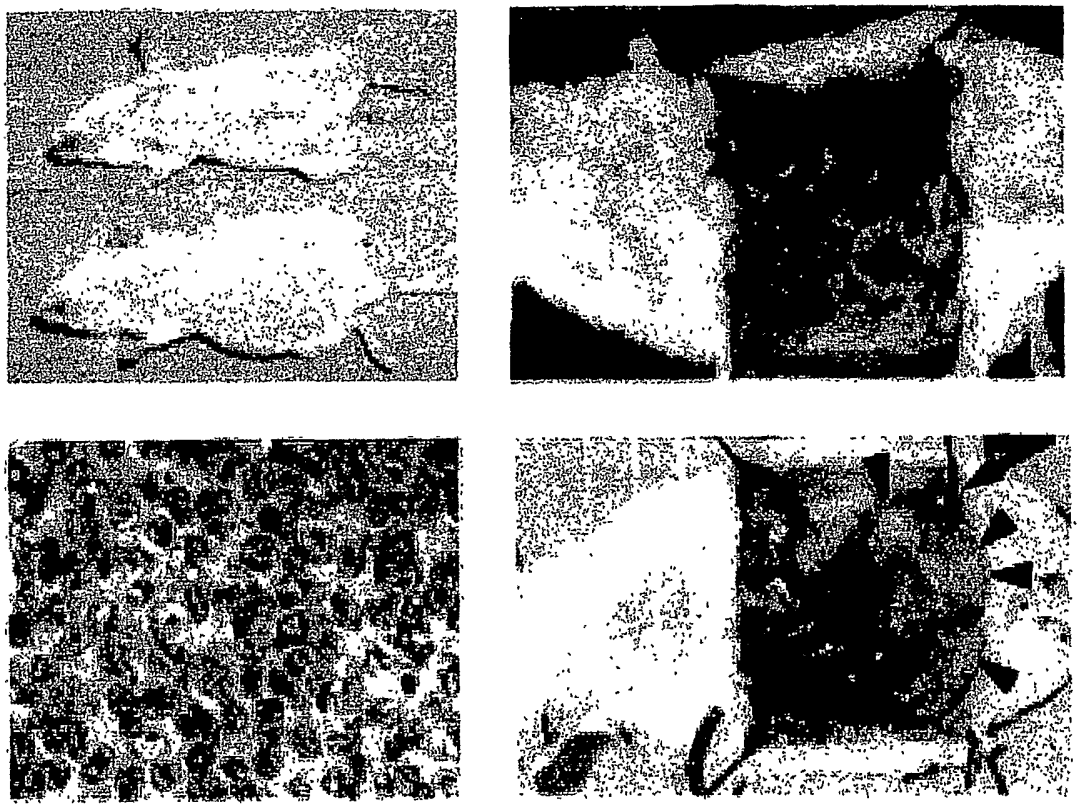


FIG. 8A

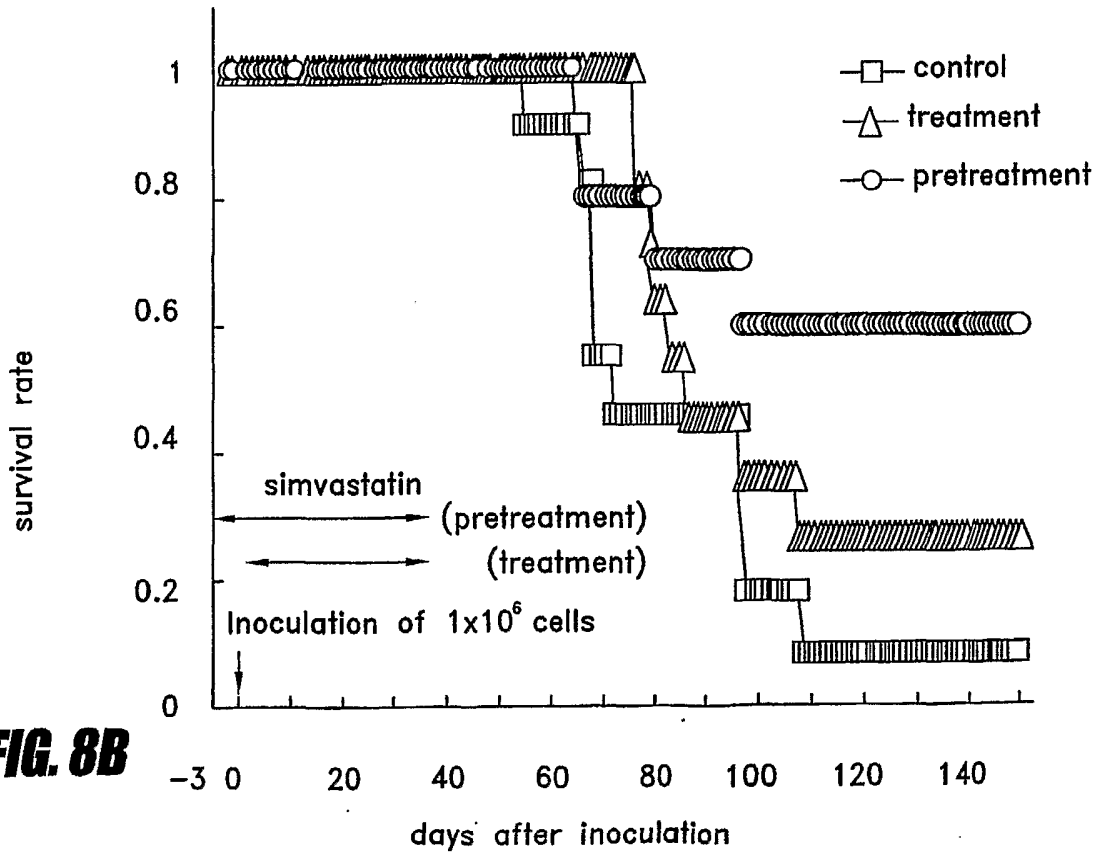
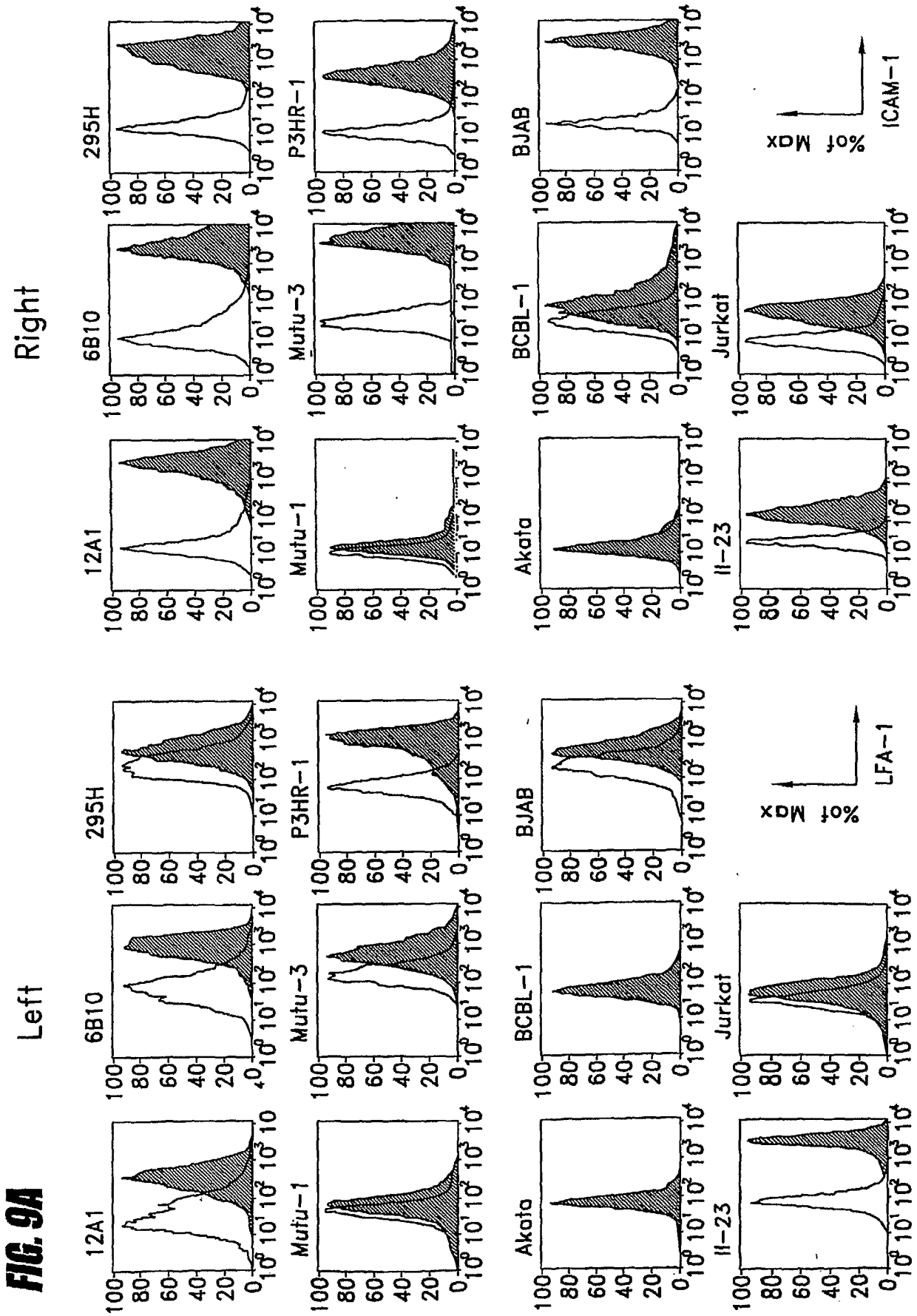
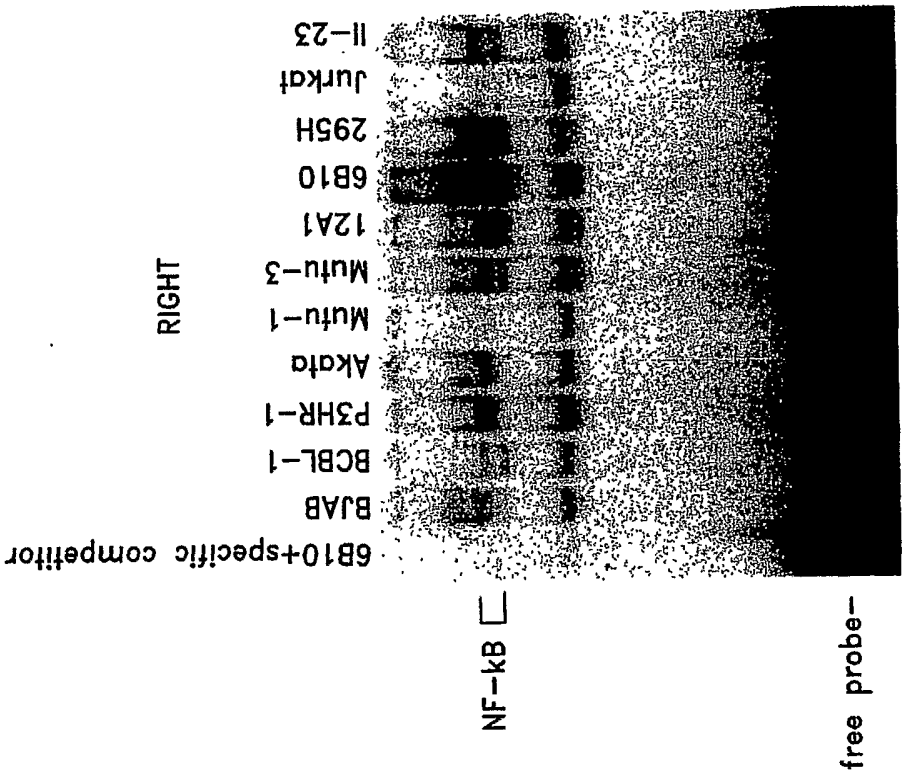

control
treatment
pretreatment
survival rate
1
0.8
0.6
0.4
0.2
0
simvastatin
(pretreatment)
(treatment)
Inoculation of 1x10^6 cells
-3 0
20
40
60
80
100
120
140
days after inoculation

FIG. 8B

FIG. 9A


Left
Right
12A1
6B10
295H
Mutu-1
Mutu-3
P3HR-1
Akata
BCBL-1
BJAB
Jurkat
Il-23
% of Max
LFA-1
ICAM-1


RIGHT
6B10+specific competitor
BJAB
BCBL-1
P3HR-1
Akata
Mutu-1
Mutu-3
12A1
6B10
293H
Jurkat
II-23
NF-kB
free probe-

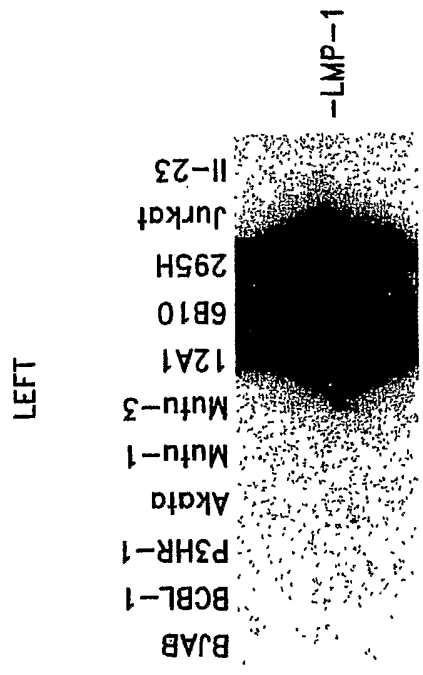

LEFT
BJAB
BCBL-1
P3HR-1
Akata
Mutu-1
Mutu-3
12A1
6B10
293H
Jurkat
II-23
-LMP-1

FIG. 9B

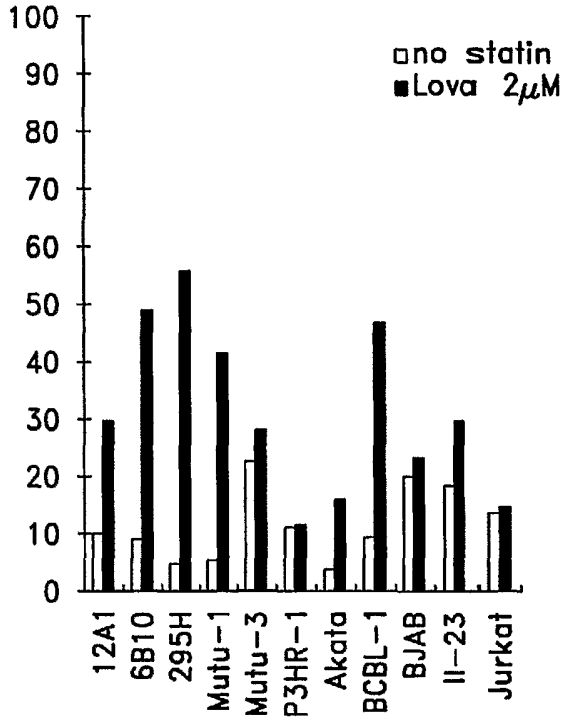


FIG. 10A

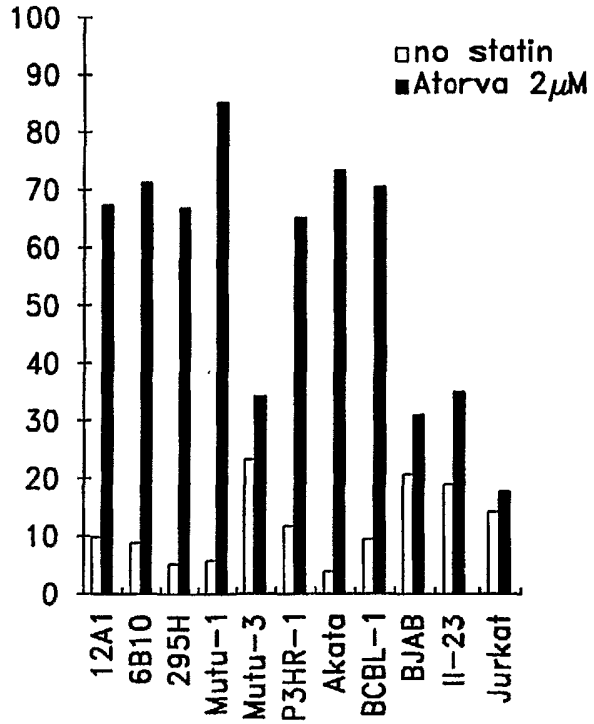


FIG. 10B

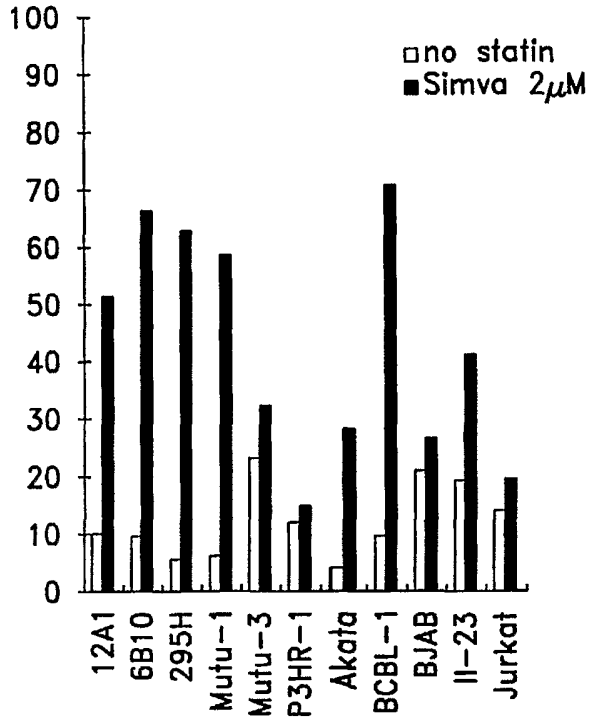

100
90
80
70
60
50
40
30
20
10
0
no statin
Simva 2μM
12A1
6B10
295H
Mutu-1
Mutu-3
P3HR-1
Akata
BCBL-1
BJAB
II-23
Jurkat

FIG. 10C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/035829

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/366 A61P31/22 A61P35/00 A61P43/00

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 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data, MEDLINE, EMBASE, BIOSIS, CANCERLIT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KATANO, H.; PESNICAK, L.; COHEN, J.I.: "Simvastatin induces apoptosis of Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines and delays development of EBV lymphomas" PNAS, vol. 101, no. 14, 6 April 2004 (2004-04-06), pages 4960-4965, XP002321100 the whole document ----- -/--	1-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2005	23/03/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Taylor, G.M.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/035829

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 99/58505 A (WARNER-LAMBERT COMPANY; LEOPOLD, JUDITH; NEWTON, ROGER, SCHOFIELD) 18 November 1999 (1999-11-18) abstract page 5, line 20 - line 27 page 20, line 26 - line 29 page 30, line 24 - page 31, line 32 page 33, line 14 - line 20 page 36, line 16 - line 19 claims 1-30	1-7,11, 12, 14-20, 23,24
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